Organizing committee
Prof. dr. M.D. de Jong, chair
Prof. dr. A.J.W. van Alphen
Prof. dr. W. Bitter
Prof. dr. S. Brul
Prof. dr. L. Dijkhuizen
Dr. B. Duim
Dr. J.W.B. van der Giessen
Dr. P.J.A. Haas
Prof. dr. ir. M.S.M. Jetten
Prof. dr. M.P.G. Koopmans
Prof. dr. O.P. Kuipers
Prof. dr. P. Rottier
Prof. dr. P.H.M. Savelkoul
Dr. B.J.M. Vlaminckx
Prof. dr. ir. M.H. Zwietering

Poster committee
Prof. dr. S. Brul
Dr. W. van Schaik
Dr. A.M.J. Wensing

The Scientific spring meeting is organized by the Dutch Society of Medical Microbiology (NVMM) and the Royal Dutch Society of Microbiology (KNVM).

NWO
Netherlands Organisation for Scientific Research

Meeting secretariat
Congress Company

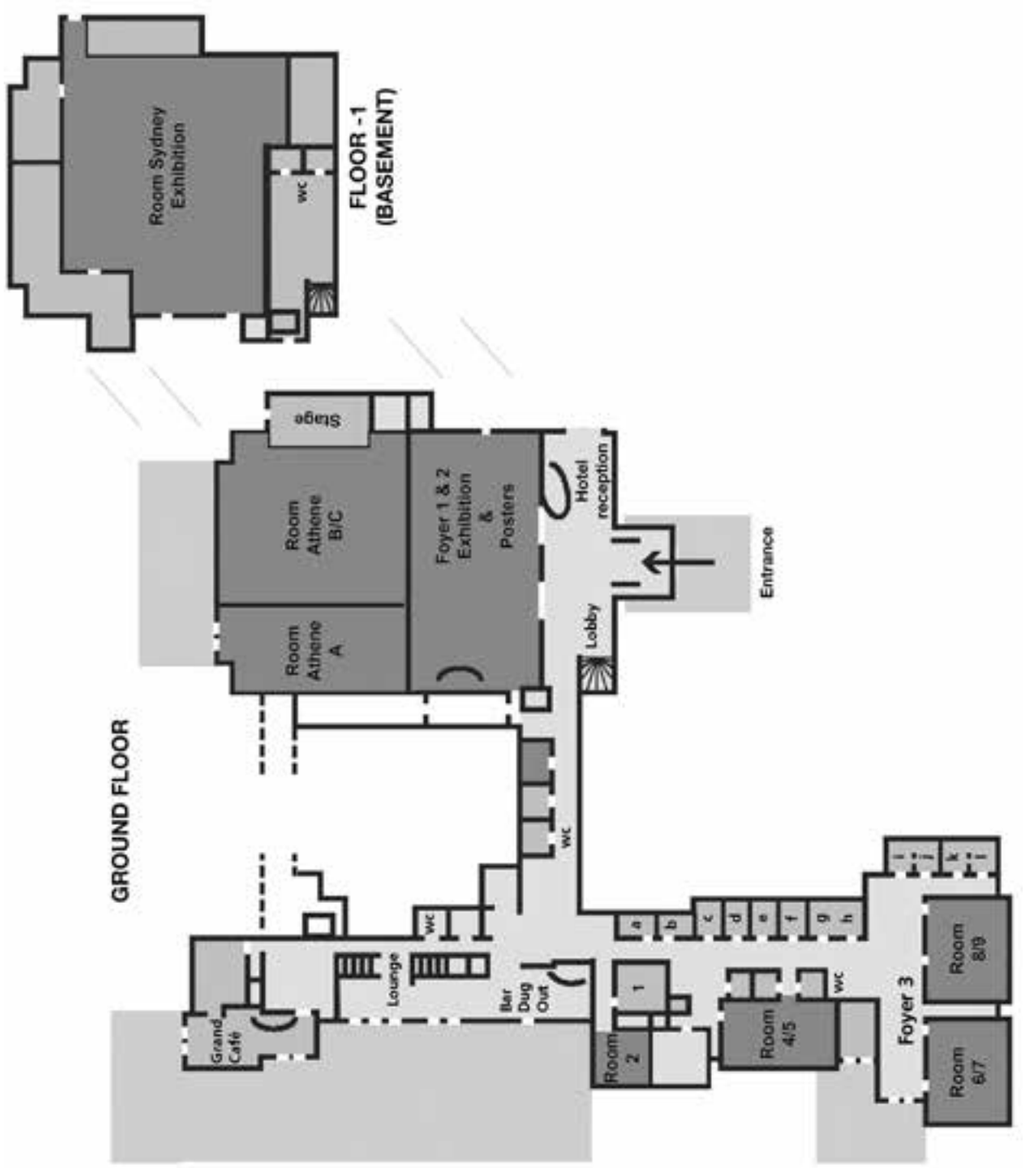
congress company

P.O. Box 2428
5202 CK 's-Hertogenbosch
Tel 073 - 700 35 00
info@congresscompany.com
www.congresscompany.com

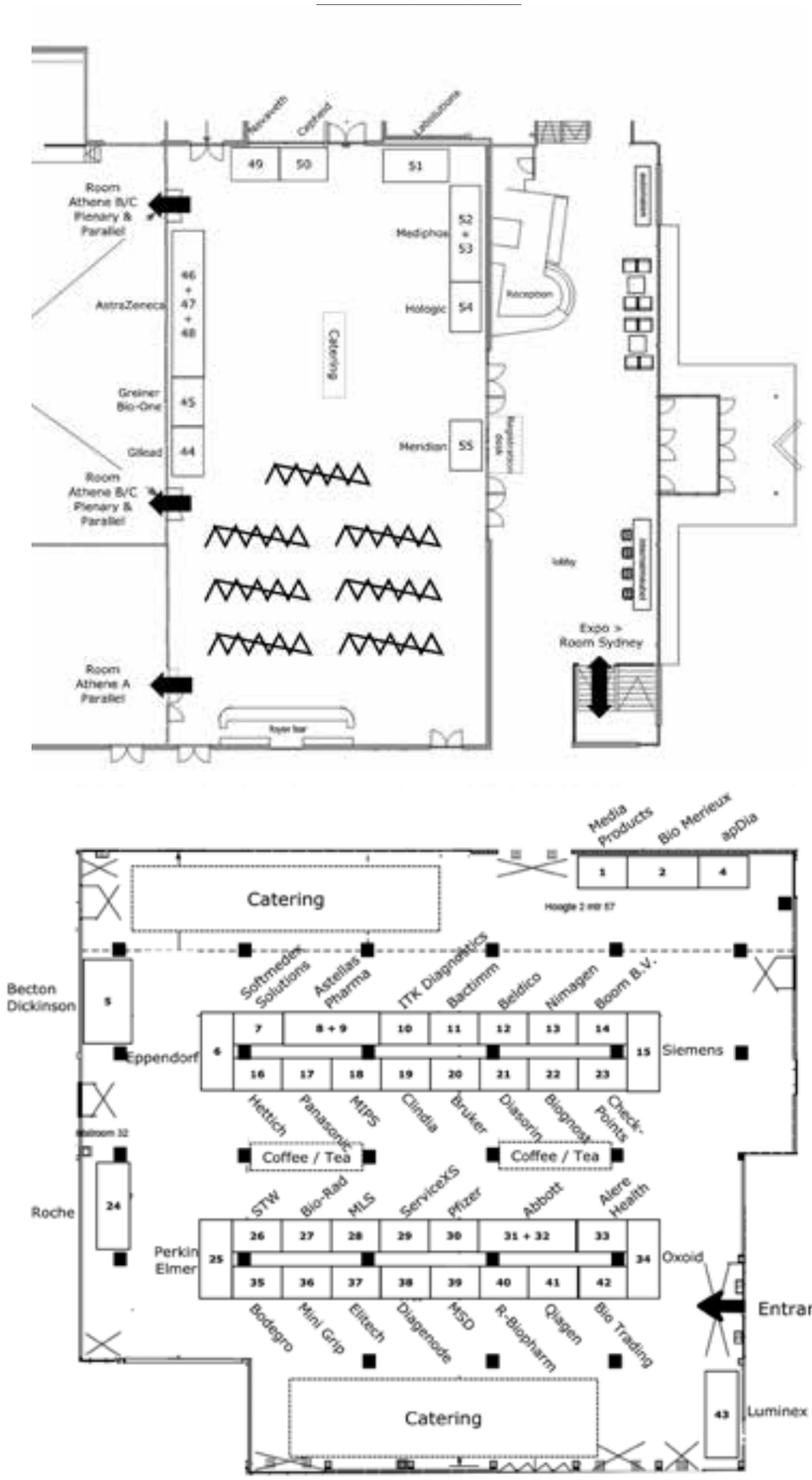
TUESDAY APRIL 16, 2013							
	EXHIBITION	ROOM ATHENE B/C	ROOM ATHENE A	ROOM 2	ROOM 4/5	ROOM 6/7	ROOM 8/9
09:00 - 09:30	Registration						
09:30 - 11:00		Plenary session					
11:00 - 11:30	Coffee/tea						
11:30 - 12:45		Plenary session & Award Ceremony					
12:45 - 14:00	Lunch		KNVM Business Meeting				
14:00 - 15:30		New challenges in infection prevention	Community acquired *Clostridium difficile* infections: from pigs to humans and vice versa	Leishmaniasis: a curable disease?	Intestinal microbes and host interplay	Microbial pathogenesis 1	Clinical microbiology WAMM: Back to the roots - diagnostics of urinary tract infections
15:30 - 16:00	Coffee/tea						
16:00 - 17:30		Clinical microbiology	Bacterial cell growth	Campylobacter – host interactions	Clinical development of malaria drugs and vaccines in the Netherlands	Highly resistant micro-organisms outside the hospital - What can we expect from nursing homes and public health services?	STD dynamics in the Netherlands and Europe
17:30 - 18:30	Drinks						
18:30 - 20:30		Dinner					
20:30 - 22:15		Poster session & Poster award ceremony					
22:15 - 01:30			Party				

WEDNESDAY APRIL 17, 2013							
	EXHIBITION	ROOM ATHENE B/C	ROOM ATHENE A	ROOM 2	ROOM 4/5	ROOM 6/7	ROOM 8/9
08:30 - 09:00	Registration						
09:00 - 10:30		Viral zoonoses	Whole genome sequencing in clinical microbiology and its applications in understanding microbial biology	Antimicrobial peptides: a rich source for developing novel antibiotics	NVMy symposium 1: Detection and diagnosis	New human and veterinary vaccines against tuberculosis	Finding the needle in the haystack: molecular diagnostics of gastro enteritis
10:30 - 11:00	Coffee/tea						
11:00 - 12:30		NVMy symposium 2: Epidemiology and treatment	Virology	Microbial physiology	Computer assisted teaching	Experimental evolution	Microbial pathogenesis 2
12:30 - 14:00	Lunch						
13:00 - 14:00			BBC-MMO Business Meeting				
14:00 - 15:30		WMDI: Novel approaches in the detection of antimicrobial resistance	Antimicrobial resistance	Horen, zien en vooral niet zwijgen (NL-talige sessie)	Travel-related diseases	Genome-associated biomarkers for vaccines	Progress in microbiology
15:30 - 16:00	Coffee/tea						
16:00 - 17:30		NVMM Business Meeting					

FLOORPLAN PAPENDAL



EXHIBITION ROOMS



MAJOR SPONSOR

AstraZeneca

SPONSORS AND EXHIBITORS

Abbott Diagnostics
Alere Health
ApDia
Astellas Pharma
Bactimm
Baseclear
Becton Dickinson
Beldico
Bio Rad Laboratories
Bio Trading Benelux
Biognost
BioMerieux Benelux
Bodégro
Boom
Bruker Nederland
Cepheid Benelux
Check-Points
Clindia Benelux
Diagenode Diagnostics
DiaSorin
Elitech Benelux
Eppendorf Nederland
Gilead Sciences Netherlands
Greiner Bio-One
Hettich Benelux
Hologic Netherlands
ITK Diagnostics
Labolutions
Luminex
Mediaproducts
Mediphos Medical Supplies
Merck Sharp & Dohme
Meridian Bioscience
Minigrip Nederland
MIPS
MLS
MP Products
Nimagen
Novaveth
Oxoid
Panasonic Biomedical Sales Europe
PerkinElmer
Pfizer
Qiagen Benelux
R-Biopharm AG
Roche Diagnostics Nederland
ServiceXS
Siemens Healthcare Diagnostics
Softmedex Solutions
Technologiestichting STW
Unilever Vlaardingen

SCIENTIFIC PROGRAMMA

MONDAY 15 APRIL 2013

Room Athene A

13:00 - 17:15 **National examination for medical microbiologists in training**

Restaurant

19:00 - 21:00 **Dinner**

TUESDAY 16 APRIL 2013

09:00 - 09:30 Registration

09:30 - 11:00 Plenary session

Athene B/C *Chair: Oscar Kuipers*

09:30 - 10:15 **c-di-AMP, an essential signaling nucleotide in Gram-positive bacteria**

O001 Prof. Jörg Stülke, Institut für Mikrobiologie und Genetik, Göttingen, Germany

10:15 - 11:00 **From antigenic variation to base J in trypanosomatids**

O002 Prof. Piet Borst, NKI, Amsterdam

11:00 - 11:30 Coffee/tea break

11:30 - 12:45 Plenary session & award ceremony

Athene B/C *Chair: Menno de Jong*

11:30 - 12:15 **Host switches and evolution: news from viral reservoir investigations**

O003 Prof. Christian Drosten, Institute of Virology – University of Bonn Medical Centre, Bonn, Germany

12:15 - 12:45 **Award ceremony**

12:45 - 14:00 Lunch

Athene A

13:00 - 14:00 NVvM Business meeting

14:00 - 15:30 Parallel sessions

Athene B/C **New challenges in infection prevention**

Chair: Greet Vos

14:00 - 14:30 **A nosocomial outbreak of VIM-positive *Pseudomonas aeruginosa*: problems and pitfalls**

O004 Juliette Severin

14:30 - 15:00 **From guidelines to implementation: do we need fasces?**

O005 Jan Kluytmans

15:00 - 15:30 **Infection risks in hospitals: it's the environment stupid!**

O006 Jan van Zeijl

Athene A **Community acquired *Clostridium difficile* infections: from pigs to humans and vice versa**

Chair: Ed Kuijper

14:00 - 14:30 ***Clostridium difficile* infections outside healthcare facilities in Denmark**

O007 Katharina Olsen (Denmark)

14:30 - 14:45 ***Clostridium difficile* 078 in pigs, a threat for farmers and employees**

O008 Liny Keessen

14:45 - 15:00 **Whole-genome sequencing reveals potential interspecies transmission of *Clostridium difficile* type 078**

O009 Wilco Knetsch

15:00 - 15:15 **Outbreaks of *Clostridium difficile* type 027 infections in nursing homes; tip of the iceberg?**

O010 Sofie van Dorp

15:15 - 15:30 **Micro-array analysis and phenotypic characterization of *Clostridium difficile* cell membrane protease knock-out strains: reveals a possible link to virulence**

O011 Dennis Bakker

Room 2 **Leishmaniasis: a curable disease?**

Chair: Titia Kortbeek

14:00 - 14:30 **Asymptomatic carriers in visceral leishmaniasis**

O012 Epco Hasker (Belgium)

14:30 - 15:00 **Effectiveness of treatment of visceral leishmaniasis**

O013 Koert Ritmeijer

15:00 - 15:15 **Therapy for leishmaniasis in returning travellers**

O014 Caspar Hodiamont

15:15 - 15:30 **Leishmaniasis in the Netherlands 2005-2012: epidemiology, diagnostic techniques and sequence-based species typing in 192 patients**

O015 Aldert Bart

Room 4/5 **Intestinal microbes and host interplay**

Chairs: Peter van Baarlen & Clara Belzer

14:00 - 14:30 **Adaptive immunity, microbiota composition and prevention of pathobiont outgrowth in zebrafish**

O016 Sylvia Brugman

14:30 - 15:00 **The gut microbiome using metagenomics and dedicated bioinformatics**

O017 Jeroen Raes

15:00 - 15:15 **The effect of viral, bacterial and parasitic pathogens on the intestinal microbiota**

O018 Dries Budding

15:15 - 15:30 ***Streptococcus suis* interaction with human intestinal epithelial cells**

O019 Maria Laura Ferrando

Room 6/7 **Microbial pathogenesis 1**

Chair: Pieter-Jan Haas

14:00 - 14:15 **The Staphylococcal Panton-Valentine Leukocidin targets C5a receptors**

O020 András Spaan

14:15 - 14:30 **Pneumococcal meningitis: heterogenous receptor expression and the influence on *Streptococcus pneumoniae* interaction with the blood-brain barrier**

O021 Federico Iovino

14:30 - 14:45 ***Staphylococcus aureus* secretes extracellular adherence proteins that block neutrophil serine proteases (NSPs)**

O022 Daphne Stapels

14:45 - 15:00 **Distinct localization and assembly of complement C5b-9 on gram-positive bacteria**

O023 Evelien Berends

15:00 - 15:15 **Identification of novel factors affecting *Moraxella catarrhalis* adhesion**

O024 Stefan de Vries

15:15 - 15:30 **Phosphate starvation induces the expression of a *Pseudomonas aeruginosa* ECF sigma factor and activates a virulence phenotype**

O025 Karlijn Bastiaansen

Room 8/9 **Clinical microbiology**

WAMM: Back to the roots – diagnostics of urinary tract infections

Chair: Rolf Vreede

14:00 - 14:30 **Urinary tract infections: old and new diagnostic possibilities**

O026 Niek Arents

14:30 - 14:45 **Everything you always wanted to know about urine cultures, but were afraid to ask**

O027 Caroline Visser

14:45 - 15:00 **A multitarget qPCR assay for the diagnosis of bacterial vaginosis**

O028 Wendelien Dorigo-Zetsma

15:00 - 15:15 **Urosepsis due to *Actinobaculum schaalii*: first case series from the Netherlands**

O029 Daphne Scoop

15:15 - 15:30 **Predicting outcome of urine cultures by automated urine microscopy – results of a pilot study**

O030 Elske Kusters

15:30 - 16:00 **Coffee/tea break**

16:00 - 17:30 **Parallel sessions**

Athene B/C **Clinical microbiology**

Chair: Wim Ang

16:00 - 16:15 **Large outbreak of *Salmonella* Thompson related to smoked salmon**

O031 Ingrid Friesema

16:15 - 16:30 **Molecular epidemiology of an epidemic rise of vancomycin-resistant *Enterococcus faecium* in the Netherlands**

O032 Jan Sinnige

16:30 - 16:45 ***Yersinia pseudotuberculosis* as cause of terminal ileitis without diarrhea in three patients**

O033 Herman Wunderink

16:45 - 17:00 **Identification of a new biomarker for fast discrimination between epidemic *V. cholerae* O1/O139 and non-epidemic *V. cholerae* in a modified MALDI-TOF MS assay**

O034 Armand Paauw

17:00 - 17:15 **Airway microbiome in Cystic Fibrosis patients: exploring culturing bias**

O035 Jorrit Jan Hofstra

17:15 - 17:30 **Urinary antigen test established pneumococcal pneumonia has same outcome as bacteraemic pneumococcal pneumonia**

O036 Suzan van Mens

Athene A **Bacterial cell growth**

Chair: Eefjan Breukink

16:00 - 16:30 **Regulation of bacterial cell wall synthesis**

O037 Waldemar Vollmer

16:30 - 17:00 **Analysis and control of *Streptomyces* morphology in liquid-grown cultures**

O038 Dennis Claessen

17:00 - 17:15 **Exogenous LPXTG containing peptides incorporation in *Staphylococcus aureus* cell wall in situ in sortase A- and growth phase dependent manner**

O039 Silvie Hansenova Manaskova

17:15 - 17:30 **The role of glycosyl-hydrolases in antibiotic subsistence**

O040 Teresita de Jesus Bello Gonzalez

Room 2 **Campylobacter – host interactions**

Chair: Rogier Louwen

16:00 - 16:30 ***Campylobacter jejuni* induces acute enterocolitis in gnotobiotic IL-10-/- mice via Toll-like-receptor-2 and -4 signaling**

O041 Markus Heimesaat

16:30 - 17:00 **Host-pathogen interactions in Guillain-Barré syndrome**

O042 Astrid Heikema

17:00 - 17:15 **Dsb system of *Campylobacter jejuni* influences γ-glutamyltranspeptidase activity by altering the status of the RacRS two-component system**

O043 Anne-Xander van der Stel

17:15 - 17:30 **A novel link between *Campylobacter jejuni* bacteriophage defence, virulence and Guillain-Barré syndrome**

O044 Rogier Louwen

Room 4/5 **Clinical development of malaria drugs and vaccines in the Netherlands**

Chair: Robert Sauerwein

16:00 - 16:30 **Dutch contribution to malaria vaccine and novel drug development**

O045 Robert Sauerwein

16:30 - 16:45 **Preclinical development of genetically attenuated malaria parasites for vaccine development**

O046 Shahid Khan

16:45 - 17:00 **Preclinical development of malaria drugs against liver stages**

O047 Clemens Kocken

17:00 - 17:15 **Efficacy and safety of ivermectin to prevent malaria transmission after treatment of *Plasmodium falciparum* infections with artemether-lumefantrine: a double-blind randomized controlled clinical trial**

O048 Guido Bastiaens

17:15 - 17:30 **Complete protection against malaria after sporozoite immunization of volunteers under chloroquine prophylaxis is mediated by pre-erythrocytic immunity**

O049 Else Bijker

Room 6/7 **Highly resistant microorganisms outside the hospital. What can we expect from nursing homes and public health services?**

Chairs: Bartelt de Jongh & Ellen Stobberingh

16:00 - 16:30 **HRMO and other infectious diseases outbreaks in nursing homes**

O050 Wilco Achterberg

16:30 - 16:45 **HRMO – what can you expect from Municipal Health Services?**

O051 Anja Schreijer

16:45-17:00 **MRSA *spa* t1081 preferentially affects long term care facilities**

Paul Gruteke

17:00 - 17:15 **Holistic risk assessment identifies large variation in infection control practices and outcome in Dutch nursing homes**

O052 Ina Willemsen

17:15 - 17:30 **National surveillance of carbapenemase producing *Enterobacteriaceae* in the Netherlands 2011-2012**

O053 Daan Notermans

Room 8/9 **STD dynamics in the Netherlands and Europe**

Chair: Christian Hoebe

16:00 - 16:30 **Chlamydia dynamics in Europe**

O054 Nicola Low (Switserland)

16:30 - 16:45 **Human papilomavirus (HPV) dynamics in the Netherlands**

O055 Marianne van der Sande

16:45 - 17:00 **The dynamics of gonorrhoea in the Netherlands**

O056 Jan van Bergen

17:00 - 17:15 **False-positive *Neisseria gonorrhoeae* results in urine samples using a highly sensitive NAAT tests, resulting in a pseudo-outbreak of gonorrhoea**

O057 Alje van Dam

17:15 - 17:30 **Low prevalence after systematic screening for *Trichomonas vaginalis* in three patient cohorts from general practitioners, STI clinic and a national population based Chlamydia screening study**

O058 Tanja Geelen

Room Sydney

17:30 - 18:30 **Drinks**

Restaurant

18:30 - 20:30 **Dinner**

Foyer

20:30 - 22:00 **Poster session**

22:00 - 22:15 **Poster award ceremony**

Athene A

22:15 - 01:30 **Party**

WEDNESDAY 17 APRIL 2013

08:30 - 09:00 Registration

09:00 - 10:30 **Parallel sessions**

Athene B/C **Viral zoonoses**

Chair: Rik de Swart

09:00 - 09:30 **Paramyxoviruses crossing the species barrier: a continuing story**

O060 Ab Osterhaus

09:30 - 10:00 **Mapping the molecular events during adaptation of canine distemper virus to primates**

O061 Paul Duprex (USA)

10:00 - 10:15 **Dipeptidyl peptidase-4 is a functional receptor for the emerging human coronavirus-EMC**

O062 Huihui Mou

10:15 - 10:30 **Ferreting out influenza H5N1 quasispecies evolution during human infection by whole genome deep sequencing of clinical specimens from infected patients**

O063 Matthijs Welkers

Athene A **Whole genome sequencing in clinical microbiology and its applications in understanding microbial biology**

Chair: Mark de Been

09:00 - 09:30 **The use of whole-genome sequence data to detect recent homologous recombination event in *Enterococcus faecium***

O064 Mark de Been

09:30 - 10:00 **Utility of whole genome sequencing of *Mycobacterium tuberculosis* in the molecular epidemiology of tuberculosis**

O065 Dick van Soolingen

10:00 - 10:30 **Next generation sequencing to elucidate the molecular epidemiology of pathogens**

O066 Alexander Mellman (Germany)

Room 2 **Antimicrobial peptides: a rich source for developing novel antibiotics**

Chair: Oscar Kuipers

09:00 - 09:30 ***Streptomyces*: the beauty of the beast and its exploitation for the discovery of novel antimicrobials**

O067 Gilles van Wezel

09:30 - 10:00 **Synthetic Biology applied to the discovery and improvement of lantibiotics**

O068 Manolo Montalbán-López

10:00 - 10:15 **Chicken cathelicidins display antimicrobial activity against multiresistant bacteria without inducing strong resistance**

O069 Edwin Veldhuizen

10:15 - 10:30 **Real-time *in vivo* imaging of invasive- and biomaterial-associated bacterial infections using fluorescently labeled vancomycin**

O070 Marleen van Oosten

Room 4/5 **NVMy symposium 1: Detection and diagnosis**

Chairs: Tjomme van der Bruggen & Sybren de Hoog

09:00 - 09:30 **The fungi strikes back: multidrug resistance in Aspergillus fumigates and agricultural use of fungicides**

O071 Willem Melchers

09:30 - 10:00 **Chest imaging and detection and diagnosis of invasive fungal disease in the immuno-compromised host**

O072 Pim de Jong

10:00 - 10:15 **Diagnosis of *Pneumocystis jirovecii* pneumonia (PJP) and differentiation between active PJP and colonization in immunocompromised patients with real-time PCR**

O073 René te Witt

10:15 - 10:30 **The classical complement pathway induces phagocytosis of *Aspergillus fumigatus***

O074 Steven Braem

Room 6/7 **New human and veterinary vaccines against tuberculosis**

Chair: Jelle Thole

Human TB vaccines:

09:00 - 09:30 **Global progress in TB vaccine development**

O075 Helen McShane (United Kingdom)

09:30 - 09:45 **From *Mycobacterium tuberculosis* antigen discovery to new TB subunit vaccines in humans**

O076 Tom Ottenhoff

Veterinary TB vaccines:

09:45 - 10:15 **TB vaccines for badgers and cattle**

O077 Glyn Hewinson (United Kingdom)

10:15 - 10:30 **Post-exposure subunit vaccination against chronic mycobacterial infection in a natural host**

O078 Ad Koets

Room 8/9 **Finding the needle in the haystack: molecular diagnostics of gastro enteritis**

Chairs: Alexander Friedrich & Titia Kortbeek

09:00 - 09:15 **STEC-ID-net: a feasibility study for a new STEC diagnostic strategy**

O079 Mirjam Kooistra-Smid

09:15 - 09:30 **CCGE Study gastro-enteritis – first results**

O080 Lesla Bruijnesteijn van Coppenraet

09:30 - 09:45 **Molecular diagnostics of intestinal parasites**

O081 Theo Schuurs

09:45 - 10:00 **Molecular diagnostics of intestinal parasites; implications of the introduction in routine clinical practice**

O082 Theo Mank

10:00 - 10:15 **Direct molecular analysis of polymicrobial infections by endogenous microbiota with IS-pro**

O083 Martine Hoogewerf

10:15 - 10:30 **Discussion: Implications of introduction of molecular diagnostics**

10:30 - 11:00 **Coffee/tea break**

11:00 - 12:30 **Parallel sessions**

Athene B/C **NVMy symposium 2: Epidemiology and treatment**

Chairs: Ferry Hagen & Paul Verweij

11:00 - 11:30 **Prevention of invasive fungal infections: evidence, epidemiology and risk profiles as a guide**

O084 Bart Rijnders

11:30 - 12:00 **tba**

O085 William Hope (United Kingdom)

12:00 - 12:15 **Exposure of *Aspergillus fumigatus* to methylprednisolone results in increased expression of cell wall genes associated with virulence**

O086 Erik Bathoorn

12:15 - 12:30 **Caspofungin does not skew the early cytokine balance in experimental invasive pulmonary Aspergillosis**

O087 Jeannine Refos

Athene A **Virology**

Chair: Ann Vossen

11:00 - 11:30 **HEV infection among Dutch blood donors, 1988-2012**

O088 Hans Zaaijer

11:30 - 11:45 **HEV infection post allogenic hematopoietic stem cell transplantation recipients may be misdiagnosed as graft versus host disease or drug toxicity**

O089 Suzan Pas

11:45 - 12:00 **Lack of X4-tropic HIV prevents viral rebound post CCR5-fj32 stem Cell Transplantation in the 'Berlin Patient'**

O090 Jori Symons

12:00 - 12:15 **The antipicornaviral activity of the antifungal drug itraconazole**

O091 Lonneke van der Linden

12:15 - 12:30 **Association between BK polyomavirus serostatus and post transplantation viremia in a kidney transplant cohort of living related donor-recipient pairs**

O092 Herman Wunderink

Room 2 **Microbial physiology**

Chairs: Stanley Brul & Marcel Zwietering

11:00 - 11:15 **Noise promotion of autorepressed AbrB by a small regulatory RNA**

O093 Ruben Mars

11:15 - 11:30 **A combination of genome-scale analyses and evolutionary engineering of butanol tolerance in *Saccharomyces cerevisiae* reveal an essential role of protein degradation**

O094 Daniel González Ramos

11:30 - 11:45 **Construction and characterization of a synchronized bacterial oscillator**

O095 Brendan Ryback

11:45 - 12:00 **Dissection of yeast responses to extreme calorie restriction and energy starvation**

O096 Pascale Daran-Lapujade

12:00 - 12:15 **Genome mining of the rhizosphere bacterium *Pseudomonas* sp. SH-C52**

O097 Menno van der Voort

12:15 - 12:30 **Stringent response activation via phosphate stress modulates *Mycobacterium tuberculosis* capsular components a-glucan and arabinomannan**

O098 Robert van de Weerd

Room 4/5 **Computer-assisted teaching**

Chair: Loek van Alphen

11:00 - 11:15 **Improved quality using less teachers**

O099 Han Wösten

11:15 - 11:45 **Computer-assisted teaching in the UK and US: @10queues - #adaptordie**

O100 Paul Duprex (USA)

11:45 - 12:00 **App-lication of tablets in teaching**

O101 Nico Boot

12:00 - 12:30 **Apps for labs**

O102 Koos van der Kolk

Room 6/7 **Experimental evolution**

Chair: Svetlana Alexeeva

11:00 - 11:30 **Genome evolution in a long-term experiment with *Escherichia coli***

O103 Dominique Schneider

11:30 - 12:00 **Evolution in synthetic microbial gene networks**

O104 Sander Tans

12:00 - 12:15 **Evolutionary adaptation of *Akkermansia* species within the mammalian host**

O105 Janneke Ouwerkerk

12:15 - 12:30 **Experimental evolution in traditional fermented products**

O106 Sijmen Schoustra

Room 8/9 **Microbial pathogenesis 2**

Chair: Wilbert Bitter

11:00 - 11:15 ***Staphylococcus aureus* biofilm matrix does not hide the bacteria, but instead strongly activates the innate immune system**

O107 Reindert Nijland

11:15 - 11:30 **AtlAEfm: The major autolysin in *Enterococcus faecium* involved in cell separation, surface Acm exposure, eDNA release and biofilm formation**

O108 Fernanda Paganelli

11:30 - 11:45 **The papain inhibitor (SPI) of *Streptomyces mobaraensis* inhibits bacterial cysteine proteases and is an antagonist of bacterial growth**

O109 Wendy Kaman

11:45 - 12:00 **The ESX-5 secretion system is essential for *Mycobacterium marinum* viability because it controls the permeability of the mycobacterial outer membrane**

O110 Louis Ates

12:00 - 12:15 **Disulfide bond formation proteins are essential for complement-resistance of *Moraxella catarrhalis***

O111 Stefan de Vries

12:15 - 12:30 **The role of staphylococcal PSMs in survival and killing within neutrophils**

O112 Bas Surewaard

12:30 - 14:00 **Lunch**

Athene A

13:00 - 14:00 **BBC-MMO Business meeting**

14:00 - 15:30 **Parallel sessions**

Athene B/C **WMDI: Novel approaches in the detection of antimicrobial resistance**

Chairs: Ed Kuijper & John Rossen

14:00 - 14:30 **Molecular genetics, epidemiology and biochemistry of emerging antibiotic resistance mechanisms**

O113 Thierry Naas (France)

14:30 - 15:00 **A next generation mass-spectrometry platform for the rapid identification of (multi-)drug resistant gram-negative bacteria**

O114 Paul Hensbergen

15:00 - 15:15 **Characterization of antibiotic resistance genes from a metagenomic library from a human gut microbiota enrichment culture**

O115 Teresita de Jesus Bello Gonzalez

15:15 - 15:30 **Evaluation of the efficacy of bacteriophages-derived lytic enzymes (lysins) to reduce colonization of *Streptococcus suis* in pigs**

O116 Niels Dekker

Athene A **Antimicrobial resistance**

Chair: Christina Vandenbroucke-Grauls

14:00 - 14:15 **Transcriptional termination regulates the expression of the multidrug-ABC-transporter BmrC/BmrD**

O117 Ewoud Reilman

14:15 - 14:30 **A novel phenotypic detection strategy for class A, B and OXA-48 carbapenemases in *Enterobacteriaceae* using temocillin**

O118 Karin van Dijk

14:30 - 14:45 **Emerging mupirocin resistance in staphylococci following the implementation of a new *S. aureus* decolonization strategy**

O119 David Hetem

14:45 - 15:00 **Prevalence of extended spectrum beta-lactamase-producing *Escherichia coli* in people living and/or working on Dutch broiler farms**

O120 Patricia Huijbers

15:00 - 15:15 **The zebrafish embryo as a novel vertebrate model for the *in vivo* analysis of biomaterial associated infection and immune responses**

O121 Oliver Stockhammer

15:15 - 15:30 **The nature and origin of resistance to antituberculosis drugs in the Netherlands in the period 1993-2011**

O122 Carolien Ruesen

Room 2 **Horen, zien en vooral niet zwijgen (Nederlandstalige sessie)**

Chair: Eric van der Vorm

14:00 - 14:30 **DICA: Verbeteren van kwaliteit van zorg door clinical auditing**

O123 Nicolien van Leersum

14:30 - 14:45 **Kwaliteit in infectiepreventie: wat te doen en hoe verder**

O124 Greet Vos

14:45 - 15:00 **Algemene visitatie commissie NVMM: horen en zien, vertrouwelijkheid, hoe dit te combineren met voldoende openheid?**

O125 Kees Verduin

15:00 - 15:15 **CCKL audits en beoordeling van kwaliteitssystemen van Nederlandse microbiologische laboratoria door de Raad van Accreditatie (RvA): 'lessons learned' en toekomstige uitdagingen voor artsen-microbioloog en laboratorium managers**

O126 Gunnar Andriesse

15:15 - 15:30 **Wat maakt een audit of visitatie succesvol?**

O127 Ed Wieles

Room 4/5 **Travel-related diseases**

Chair: Chantal Reusken

14:00 - 14:30 **Globally mobile populations in Europe and spread of communicable diseases**

O128 Philippe Gautret (France)

14:30 - 15:00 **Infectious diseases in a borderless world**

O129 Leo Visser

15:00 - 15:15 **Monitoring changes in the resistome of travellers**

O130 Christian von Wintersdorff

15:15 - 15:30 **Travelers as part of an arbovirus sentinal surveillance system; a feasibility study using 10 years of dengue diagnostics data**

O131 Natalie Cleton

Room 6/7 **Genome-associated biomarkers for vaccines**

Chair: Ben van der Zeist

14:00 - 14:30 **Biomarkers for tuberculosis vaccines**

O132 Helen Fletcher (United Kingdom)

14:30 - 15:00 **Genomics of the virus-host interaction**

O133 Arno Andeweg

15:00 - 15:15 **Autotransporter platform for the development of multivalent *Salmonella*-based vaccines**

O134 Maria Daleke

15:15 - 15:30 **Non-covalently cell-bound staphylococcal proteins are candidates for active or passive immunization**

O135 Francisco Romero Pastrana

Room 8/9 **Progress in microbiology**

Chair: Ben Appelmelk

14:00 - 14:15 **The effectiveness of bacteriophages against methicillin-resistant *Staphylococcus aureus* nasal colonization in pigs *in vitro*, *ex vivo* and *in vivo***

O136 Koen Verstappen

14:15 - 14:30 **Restriction of *Mycobacterium ulcerans* to localized geographical areas: requirement for a soil-based factor for the development of new endemic regions?**

O137 Jennifer Wolfe

14:30 - 14:45 **A metatranscriptome analysis of antibiotic resistance genes in a microbial community under natural conditions**

O139 Dennis Versluis

14:45 - 15:00 **Discovery of a novel natural transformation mechanism in *Streptococcus suis***

O140 Edoardo Zaccaria

15:00 - 15:15 **Drugs acting on respiratory ATP synthesis: drug synergy and potential extension of antibacterial spectrum**

O141 Dirk Bald

15:15 - 15:30 **Discussion**

15:30 - 16:00 **Coffee/tea break**

Athene B/C

16:00 - 17:30 **NVMM Business meeting**

O001

c-di-AMP, an essential signaling nucleotide in gram-positive bacteria

J. Stülke, F. Mehne
Georg-August University Göttingen, Dept. of General Microbiology, Göttingen, Germany

Cyclic dinucleotides (c-di-AMP and c-di-GMP) act as second messengers in several bacterial species. In the last decade these messengers have attracted the attention of molecular microbiologists and there have been several approaches to uncover their signaling landscape. In many bacteria, c-di-GMP governs the lifestyle switch between biofilm formation and motility. In contrast, little is known about the function of c-di-AMP. The gram-positive model organism *Bacillus subtilis* encodes three putative diadenylate cyclases. One of them, DisA, checks DNA integrity to control cell division and sporulation. In contrast, the precise function of the other proteins, CdaA and CdaS, is still unknown. While the genes encoding the diadenylate cyclases can be deleted individually, a strain that is unable to produce c-di-AMP is not viable. Thus, c-di-AMP is the first essential signaling nucleotide discovered so far. Interestingly, c-di-AMP is also essential in important pathogens including *Listeria* monocytogenes or *Staphylococcus aureus*. The putative roles of c-di-AMP as well as the implications of essentiality will be discussed.

References

Mehne FMP, et al. J. Biol. Chem. 2013;288:2004-2017.

O002

From antigenic variation to base J in trypanosomatids

P. Borst
The Netherlands Cancer Institute, Amsterdam

In the course of studies on the mechanism of antigenic variation in African trypanosomes[2], we found that some restriction enzyme recognition sites became partially blocked when a telomeric expression site for variant surface glycoproteins (VSG) was switched off.[1]. After a protracted search, we found that the modified base responsible for the blocked restriction sites was bèta-hydroxymethyluracil, or base J, a new base in DNA.[4]. Base J is present in all kinetoplastids and it replaces about 1% of T. We have shown that base J is synthesized in 2 steps: in the first step a specific T in DNA is hydroxylated to yield hydroxymethyluracil; in the second step this HOMeU is glycosylated to yield J.
In *Leishmania* 99% of J is present in telomeric repeats[3] and for years we tried in vain to find a telomeric function for J. In the end we found that some J is also present at the transcription termination region between convergent protein-coding gene clusters. When we eliminate this internal J by genetic/biochemical trickery in *Leishmania tarentolae*, we get massive readthrough of transcription termination sites and the cells die.[5]. Although this is a nice result, there are still a lot of loose ends that I shall discuss. These include some interesting unpublished results: in collaboration with J. Korlach at Pacific Biosciences (San Francisco) we have used their SMRT sequencing technique to precisely locate J in DNA. This provides a clue how the J-insertion machinery knows which T to modify.

References

1. Bernards A, et al. Modification of telomeric DNA in Trypanosoma brucei: a role in antigenic variation? Nucl. Acids Res. 1984;12:4153-4170.
2. Borst P, et al. Molecular basis for trypanosome antigenic variation. [Review]. Cell. 1982;29;291-303.
3. Genest PA, et al. Telomeric localization of the modified DNA base J in the genome of the protozoan parasite Leishmania. Nucleic Acids Res. 2007;35:2116-2124.
4. Gommers-Ampt JH, et al. Bèta-D-glucosyl-hydroxymethyluracil: a novel modified base present in the DNA of the parasitic protozoan T. brucei. Cell. 1993;75:1129-1136.
5. Luenen H van, et al. Glucosylated hydroxymethyluracil (DNA base J) prevents transcriptional read-through in Leishmania. Cell. 2012;150:909-921.

O005

From guidelines to implementation: do we need fasces?

J.A.J.W. Kluytmans
Amphia Hospital, LMI, Breda

There are numerous guidelines on infection control in hospitals and other health care settings. The implementation of these guidelines is problematic. The reasons for this are manyfold, but one of the most important issues is the huge amount of recommendations. Health care workers cannot adhere to all existing regulations which in a way entitles them to choose their own rules. As a result the process of care varies within and between institutes and this is associated with varying levels of patient safety.
To improve the process of care and create a culture of safety, it has been suggested to use a bundle (Latin: fasces). Bundles consist of a limited number (3-5) of proven effective measures that are implemented using a zero-tolerance approach. Several examples show that this can result in significant improvement of the outcome of patients. In infection control a bundle for catheter-related bloodstream infections was associated with reduction of the infection rate which came close to zero. In surgery bundles were associated with reduction of 35-50%.
Bundles can be effective tools to implement infection control measures and create a culture of safety.

O006

Infection risks in hospitals: it's the environment stupid!

J.H. van Zeijl
Izore Centre for Infectious Diseases Friesland, Dept. of Medical Microbiology, Leeuwarden

Hospitals encounter the continuous threat of nosocomial outbreaks, which are still accompanied with increased morbidity and mortality. A new aspect of these outbreaks are non-reimbursed costs which, due to changing financial regulations, are exploding.
Infections due to highly resistant micro-organisms (HRMO) increase worldwide. International travel, with many people bridging large distances within hours or days, seems to be an important vector for the transportation of these highly resistant micro-organisms. NDM-1 containing strains for example, originating from India, are spreading rapidly around the world. On the national level, patients moving from one hospital to another can carry micro-organisms which are easily transmissible. In addition, outbreaks can also be caused by more commensal bugs like *Clostridium difficile*, or by the spreading of norovirus either introduced by patients, visitors or health care personnel.
Hospitals should comply to national infection prevention guidelines. But despite guidelines and instructions for healthcare workers, hospitals frequently report ongoing outbreaks. Non-compliance to guidelines is one cause for this, but many reports point to the role of the hospital environment as a hidden source or niche for micro-organisms which enables them to play hide and seek.
In this presentation the role of the hospital environment will be discussed. Furniture and utensils (e.g. wheelchairs and ECG-carts) as well as bed curtains can serve as vectors when not properly cleaned. No-touch room disinfection systems, which can help cleaning rooms and wards after discharge of patients colonized with HRMO's, are discussed. But in particular this presentation will focus on the contribution of toilets, sinks, faucets and surfaces in the direct surrounding of patients.
Design of new hospitals or of those that are under construction, and of all goods that come in close contact with patients, forms a major challenge in the prevention of infections in our patients.

O007

Clostridium difficile infections outside healthcare facilities in Denmark

K.E.P. Olsen[1], L.M. Søes[1], H.M. Holt[2], S. Ethelberg[3], K. Mølbak[3], B. Böttiger[4], H.V. Nielsen[1], V. Andreasen[5], M. Kemp[2]
[1]*Dept. of Microbiology and Infection control, Statens Serum Institut, Copenhagen, Denmark,* [2]*Dept. of Clinical Microbiology, Odense University Hospital, Odense, Denmark,* [3]*Dept. of Epidemiology, Statens Serum Institut, Copenhagen, Denmark,* [4]*Dept. of Virology, Statens Serum Institut, Copenhagen, Denmark,* [5]*Dept. of Science, Roskilde University, Roskilde, Denmark*

The aim of this study was to identify risk factors for *Clostridium difficile* infection (CDI) and to describe the clinical symptoms in patients who attended general practice because of gastrointestinal complaints.
Stool samples submitted from general practice on suspicion of gastro-enteritis were analysed for bacterial, viral and parasitic gastrointestinal pathogens including *C. difficile*. A matched case-control study was conducted to reveal risk factors for CDI. Covariates investigated were primarily antibiotics, other drugs, admission to hospital, various food items, contact to animals and children < 2 years. Furthermore clinical symptoms and severity of disease were evaluated. A multivariate main effects model was fitted using conditional logistic regression.
355 cases (*C. difficile* culture positive) and 455 controls (*C. difficile* culture negative) were included in the study. Age ranged from 0.25 to 94 years of age. Fifty percent of cases were < 2 years of age. In patients ≥ 2 years of age, hospitalization and beef consumption were significantly more often reported by cases compared to controls (OR 8.4; 95% confidence interval (CI) 3.1-22.8) and (OR 5.5; 95% CI 2-15.1), respectively. Phenoxymethylpenicillin, dicloxacillin and penicillins with extended spectrum were all significantly associated to CDI (OR 14.8; 95% CI 2.7-81.7) and (OR 27.4; 95% CI 3.6-211) and (OR 9.2; 95% CI 1.9-45.4), respectively. Proton pump inhibitors were not associated to CDI.
In patients ≥ 2 years of age weight loss and stool frequency ≥ 10 times a day were reported significantly more often in cases compared to controls in univariate analysis (OR 2.8; 95% CI 1.5-5.1) and (OR 3.1; 95% CI 1.7-5.9), respectively.
In patients < 2 years of age neither hospitalization nor antibiotics were associated to CDI. Apart from stomach ache no differences in clinical symptoms were found between cases and controls in patients < 2 years of age.
This study of CDI in a community setting suggests intake of beef as a possible risk factor and reveals narrow-spectrum penicillins to be significantly associated to CDI. Analysis of clinical symptoms indicates CDI to be of clinical importance with symptoms at least as severe as gastro-enteritis caused by other gastrointestinal pathogens in patients ≥ 2 years of age.

O008

Clostridium difficile 078 in pigs, a threat for farmers and employees

E.C. Keessen[1], C. Harmanus[2], M.E.H. Bos[3], W.E. Dohmen[3], D.J. Heederik[3], J.A. Wagenaar[1], E.J. Kuijper[2], L.J.A. Lipman[1]
[1]*Faculty of Veterinary Medicine, Veterinary Public Health, Utrecht,* [2]*Leiden University Medical Center, Dept. of Medical Microbiology, Leiden,* [3]*IRAS, Epidemiology, Utrecht*

Clostridium difficile type 078 is emerging in humans and animals and is currently the third most frequently found type at the National Reference Laboratory in the Netherlands. The finding of identical *Clostridium difficile* PCR ribotype 078 isolates in piglets with diarrhea and in humans with *Clostridium difficile* infection (CDI) led to the suggestion that interspecies transmission could occur. Since *C. difficile* could be easily detected in the immediate environment of pig farms, we investigated the intestinal colonization in pig farmers, their relatives and employees, and in the pigs on the farms.
Farmers and employees (55), partners (31) and children (41) living on 32 pig farms participated in the study. Participants submitted a stool sample, and veterinarians collected pooled fecal samples of 10 different wards at each farm. Fecal samples were cultured using enrichment strategies. Suspected colonies for *C. difficile* were further identified and characterized by PCR ribotyping. Antimicrobial susceptibility was examined by E-testing. Multiple-locus variable number tandem repeat analysis (MLVA) was used to investigate the genetic similarity of selected human and porcine isolates.
C. difficile was isolated from fecal samples of pigs at 31 of the 32 farms. Type 078 was the predominant ribotype at 30 of the farms positive for *C. difficile*, at 1 farm only type 045 was present. In total 14 (25%) of the farmers and employees were positive, 4 (13%) of the partners and none of the children. All 4 partners reported regular contact with the pigs. The odds ratio for colonization and daily contact with pigs versus no contact with pigs was > 2.
In total, 18 positive human samples were detected at 15 of 32 pig farms. At 2 of the 15 farms only 1 person submitted a sample, but at the other 13 farms the number of participants ranged from 2-9, with a mean of 4 and a median of 3 participants per farm. *C. difficile* was not found in family members with less than weekly contact with pigs, at the 13 farms where colonized participants were found.
All positive farmers, employees and partners worked on positive farms and corresponding ribotypes were found in the pigs and the humans. This was type 078 in the humans and pigs on 13 farms and type 045 in the farmer and the pigs on 1 farm. Application of MLVA on *C.difficile* type 045 and 078 isolates from 3 different farms, revealed genetical related complexes encompassing human and pig isolates. Human and pig isolates did not differ in susceptibility to imipenem, co-trimoxazole, erythromycin, clindamycin, tetracycline and moxifloxacin.
The intestinal carriage rate in the population of people with direct contact with pigs positive tested for *C. difficile*, is 25%. The finding of identical isolates from humans and pigs from the same farms with MLVA, indicates that transmission, either via direct contact or the environment, likely occurs. Prospective studies are needed to determine the risk for development of CDI in this population.

O009

Whole-genome sequencing reveals potential interspecies transmission of *Clostridium difficile* type 078

C.W. Knetsch[1], L. Keessen[2], M. He[3], L. Lipman[2], E.J. Kuijper[1], J. Corver[1], T.D. Lawley[3]
[1]*Leiden University Medical Center, Medical Microbiology, Leiden,* [2]*Institute for Risk Assessment Sciences, Utrecht university, Utrecht,* [3]*Wellcome Trust Sanger Institute, Bacterial Pathogenesis Laboratory, Hinxton, USA*

Objectives: *Clostridium difficile* is the main cause of antibiotic associated diarrhea in the developed world. Although primarily known as a nosocomial pathogen, *C. difficile* PCR ribotype 078 is frequently found in patients with a community-acquired infection and often found in piglets and calves. Several studies have demonstrated that piglet and human *C. difficile* strains are closely related, suggesting interspecies transmission (< ie. zoonosis). In this study, we performed whole genome sequencing and phylogenetic analysis to compare *C. difficile* type 078 isolates from piglets and humans (farmer, employees and relatives) from the same farm.
Methods: Phylogenetic single-nucleotide polymorphism (SNP) analysis was done on 31 *C. difficile* 078 isolates of which two isolates (1 pig and 1 human) were derived from the same pig farm in the Netherlands. The other 29 isolates, mostly human (n = 27), originated from various European countries (n = 9). Whole genome sequencing was done on extracted genomic DNA using Illumina Hiseq platforms. The short DNA sequence reads (~ 100 bp) were bioinformatically mapped against an improved high-quality reference genome for type 078 (strain M120), after which SNPs were called. Several tree building approaches (distance based, maximum likelihood and Bayesian) were employed to infer the SNP based phylogeny.
Results: Initially, we identified a total of 617 SNP differences for the 31 PCR RT078 strains. Next, we analyzed the 078 genomes for regions that were associated with mobility (horizontal gene transfer and/or recombination) since these events might subvert the true phylogeny. In total, 418 SNPs were clustered into regions that are associated with mobility (transposons). These SNPs were removed for further analysis, leaving 199 phylogenetic SNPs, which were used for tree building. The inferred phylogeny shows that human and pig isolates were mingled, hence no separate pig or human clusters were identified. More strikingly, we found that the human and pig isolate from the same farm, were identical to each

other: 0 SNP-differences. Overall, the tree topology shows limited geographical clustering which implies frequent long-range transmission.
Conclusion: For the first time we report whole genome SNP typing for PCR RT078 strains. Preliminary results indicate that an identical *C. difficile* strain was found in human and pig samples isolated on the same farm. This suggests interspecies transmission of a PCR RT078 strain, although we cannot rule out transmission of *C. difficile* from the environment (common source). Currently, we are sequencing more pig and human 078 isolates which originated from the same farm. These results will also be presented on the symposium.

O010

Outbreaks of *Clostridium difficile* type 027 infections in nursing homes; tip of the iceberg?

S. van Dorp, E.J. Kuijper, E.A. Verspui, W.C. van der Zwet, I. Frenay, D.W. Notermans
LUMC, Medical Microbiology, Oegstgeest

Introduction: As of 2005, outbreaks with *Clostridium difficile* PCR ribotype 027 were recognized in the Netherlands. Soon after their recognition, the Center for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) started a typing service for *C. difficile* at the Leiden University Medical Center (LUMC).
Methods: All medical microbiologists in the Netherlands were requested to send *C. difficile* samples from patients with severe CDI and from outbreaks to the Reference Laboratory. As of May 2009, sentinel surveillance was started, with PCR ribotyping performed by the LUMC. Microbiologists and members of the infection control teams collect demographical data and clinical information of the patients with microbiological proven CDI and enter the data in the Osiris system. *C. difficile* strains are characterized by PCR ribotyping, toxinotyping, presence of genes tcdA and tcdB, presence of binary toxin genes and the presence of deletions in tcdC.
Results: A significant increase of CDI in residents in nursing homes was noticed; in a 3-year period, isolates were sent to the National Reference Laboratory from 25 different nursing homes. In 2010, clonal spread of *C. difficile* associated with type 027 occurred in a regional hospital and three associated nursing homes in region A (western part of the Netherlands). All requests for CDI diagnostics were reviewed; 329 stool samples from nursing home residents were tested for CDI of which 79 (24%) were positive. Of 11 visited regional nursing homes, 3 homes suffered from high incidence rates of CDI, varying per year from 2 per 100 residents to 6 per 100 residents. In a case-control study of residents with diarrhea, the overall mortality assessed for residents with a negative CDI test was 17% within 3 months after the diarrheal episode, whereas the mortality *of C. difficile*-associated diarrhea was 35%. A similar observation was made in 2011 in region B (eastern part of the Netherlands) when clonal spread of *C. difficile* occurred in a hospital and three neighboring nursing homes; of 282 tested samples, 64 (13.2%) were positive by a rapid molecular test for type 027. After implementation of a bundle of measures in region B, the incidence of new patients with CDI decreased, which implies that application of a bundle approach is very efficient to combat CDI outbreaks. Based on these two pilot studies and data extracted from the Surveillance Network of Infectious Diseases in Nursing homes (SNIV), we expect that 20% of all nursing homes residents develop an episode of diarrhea annually of which 10% is due to C. *difficile*. For the Netherlands, we estimate an incidence of 1400 CDI episodes annually in nursing homes with a mortality of 252 residents.
Conclusion: The increasing number of CDI cases from nursing homes deserves further attention and needs the introduction of specific algorithms and appropriate diagnostic facilities in the Netherlands.

O011

Micro-array analysis and phenotypic characterization of *Clostridium difficile* cell membrane protease knock-out strains: reveals a possible link to virulence

D. Bakker[1], W.K. Smits[1], A. de Jong[2], O.P. Kuipers[2], E.J. Kuijper[1], J. Corver[1]
[1]*Leiden University Medical Center, Dept. of Medical Microbiology, Leiden,* [2]*University of Groningen, Dept. of Molecular Genetics, Groningen*

Clostridium difficile is a gram-positive spore forming rod, which can cause a wide variety of symptoms. The main virulence factors of the enteropathogenic *Clostridium difficile* are toxin A and toxin B. Besides toxin expression, other bacterial factors contribute to the pathogenicity of *C. difficile*. For survival in the host, *C. difficile* needs to overcome stress induced by the host. Recently, it has been shown that regulated intramembrane proteolysis pathways are involved in the regulation of extracytoplasmic sigma factors that are needed for the survival of *C. difficile* in the host. Bioinformatics analysis of the *C. difficile* genome has revealed homologues of RseP and HtrA, proteases which are involved in stress response pathways, like the regulated intramembrane proteolysis.
We generated isogenic knock-out mutants in the identified proteases RseP and HtrA (CD2129 and CD3284, respectively) using ClosTron technology. *Clostridium difficile* 630fjErm (wild type) and the *C. difficile* CT:CD2129 and CT:CD3284 (fjCD2129 and fjCD3284 respectively) mutants were grown under anaerobic conditions in pre-reduced brain-heart-infusion broth supplemented with yeastextract. Using assays for sporulation, adhesion and cytotoxicity we determined the phenotype of the mutants. In addition, RNA samples, taken in the logarithmic and stationary growth phase were used for micro-array analysis to determine which genes/pathways are differentially expressed in the knock-outs compared to wild type.
Micro-array analysis revealed several down-regulated genes (e.g. slpA and spoOA) and up-regulated genes (e.g. Toxin A) in the protease knock-out mutants compared to wt. Western blot analysis and real-time qPCRs confirmed the altered transcription levels of the affected genes. Sporulation assays revealed a 1.5 log reduction in formation of spores in the knock-out mutants compared to wt. The knock-out mutants, which have decreased transcription levels of slpA, showed a 1 log reduction of binding in the adhesion assay compared to wt. Furthermore, the cytotoxicity assay detected increased levels of toxins in the knock-out mutants confirming the micro-array analysis data.
Formation of spores and adhesion to human colonic cells are important for *C. difficile* to transmit and to survive in the host. Our data show that CD2129 and CD3284 may play an important role in the formation of spores and the ability to adhere to human colonic cells. Therefore, the identified proteases may be important for the pathogenicity of *C. difficile*.

O012

Asymptomatic carriers in visceral leishmaniasis

E.C. Hasker, M. Boelaert, S. Kansal
ITG Antwerpen, Public Health, Antwerpen

Introduction: Of all persons infected with the parasites causing visceral leishmaniasis (VL) usually only 10-25% progress to clinical disease. We explored datasets from three different VL endemic study populations in India and Nepal to describe patterns of markers of *Leishmania donovani* infection and clinical VL. Our total study population was made up of 32,564 individuals.
Methods: In each of the three study populations house to house surveys were conducted during which blood samples on filter paper were collected from all consenting individuals aged 2 years and above on at least two occasions. All baseline samples were tested for anti-*Leishmania* serology by direct agglutination test (DAT) and rK39 ELISA, follow-up samples were tested with both assays in two studies and only with DAT in one study. Results from successive surveys were used to identify sero-convertors among those with negative serology at baseline. Data collected during the surveys included information on episodes of clinical VL among study participants.
Results: Initial DAT sero-prevalence ranged from 6.2 to 14.8%, rK39 seroprevalence ranged from 5.9 to 16.5%. DAT titers followed a bimodal distribution, rK39 titers were uni-modally distributed. Agreement between DAT and rK39 was limited with kappa values ranging from 0.30 to 0.46. In all three populations the probability of being DAT positive increased with age. DAT sero-conversions were observed in 2.6 to 4.6% of initially sero-negatives; in all three study populations proportions of sero-convertors increased steadily with age. Clinical VL occurred mainly among children and young adults (median ages 13-19 years). Within a one year interval 24-33% of initial DAT positives and 59% of initial rK39 positives had reverted back to sero-negative; in the study with a 6-month interval between surveys 20% of DAT positives and 17% of rK39 positives had reconverted.
Discussion: Infection with *L. donovani* is assumed to be permanent but serological markers revert back to negative. Though clinical VL is more common at younger ages, we observed a steady increase with age in the frequency of sero-positivity and of sero-conversion. Individuals in endemic areas apparently experience repeated episodes of sero-positivity. This can be explained by a boosting effect upon repeated exposure to the parasite or by intermittent release of parasites in infected subjects from safe target cells. Either mechanism can lead to misclassification of infected subjects.

O013

Effectiveness of treatment of visceral leishmaniasis

K.J.L. Ritmeijer
Médecins Sans Frontières, Public Health Department, Amsterdam

Visceral leishmaniasis (VL) is a lethal vector-borne protozoal infection caused by different species of the *Leishmania* parasite, and is one of the most neglected parasitic diseases causing large scale mortality and morbidity among the poorest of the poor in the Indian subcontinent and Africa. In 98% of cases, death can be avoided by timely treatment, even in basic field circumstances.
Since the 1940s treatment with pentavalent antimonials (sodium stibogluconate – SSG) has remained the mainstay of treatment in developing countries. Treatment is prolonged, potentially toxic, very painful, and ineffective in parts of India due to resistant *L. donovani*. Conventional amphotericin B is a highly effective drug, however, in-patient care in a well-equipped hospital for 30 days is required because of the risk of potentially serious side effects, which makes it unfeasible for treatment of most patients.
In the past decade three new effective and safer treatments have been licensed: liposomal amphotericin B, miltefosine and paromomycin. All three were originally developed for other indications.
Research has mainly focused on the clinical development phase, but not on ensuring that the drugs would reach

the patients that need them after clinical development is finished. Therefore, these new drugs have remained largely inaccessible as VL control programs in the developing world are largely lacking. Research is needed into the design of cost-effective intervention strategies in order to deliver innovation to patients, taking into account their day to day reality. The ideal VL treatment should be feasible in the circumstances in which most VL patients live: in no proximity to hospitals, and in such poverty that travelling costs and prolonged absence from work are typically insurmountable obstacles to seeking treatment. Any VL treatment should preferably be a short course, easily administered in an out-patient setting, highly effective, affordable and safe. None of the currently existing VL treatment options meet these criteria, and additionally their costs form a barrier.
All drugs currently used for VL, except amphotericin B, are prone to the development of resistance, and *Leishmania* has already developed resistance to SSG in India. Combination therapy is expected to prevent the development of resistant strains, and shorten the duration of treatment, which will have a positive impact on compliance. Combination therapy will also increase efficacy, reduce side effects, be cheaper, and allow for more cost-effective treatment programs. Current research is evaluating the efficacy of different short-course drug combinations, which may result in more cost-effective treatment regimen. No combinations have yet been rolled out in treatment programmes, except SSG/paromomycin in Africa.
Although there are only few clinical studies on the efficacy of treatments for HIV/VL coinfection, it is clear that none of the currently used drugs have proven effective in HIV/VL. Patients experience multiple relapses and become eventually unresponsive to all drugs used. Enhanced toxicity of treatments is frequently experienced, and reduces treatment options further. Combination regimen should also be tested in HIV-coinfected patients, as they may improve treatment efficacy. This is deserving of urgent attention, especially in the context of the rapidly expanding HIV/VL coinfection.

O014

Therapy for leishmaniasis in returning travellers

C.J. Hodiamont[1], P.A. Kager[1], A. Bart[1], H.J.C. de Vries[1], P.P. van Thiel[1], T. Leenstra[2], P.J. de Vries[3], M. van Vugt[1], M.P. Grobusch[1], T. van Gool[1]
[1]AMC, Dept. of Medical Microbiology, Amsterdam, [2]National Institute for Public Health and the Environment (RIVM), Bilthoven, [3]Tergooiziekenhuizen, Division of Internal Medicine, Hilversum

Introduction: Leishmaniasis is increasingly reported among travellers. Three syndromes are distinguished: visceral (VL), cutaneous (CL) and mucocutaneous leishmaniasis (MCL). *Leishmania* species vary in sensitivity to available drugs. Molecular species identification is increasingly available and will make species-specific treatment possible. To develop guidelines for species-specific treatment of leishmaniasis has proven to be difficult. Absence of parasitological confirmation and species characterization, lack of clearly defined treatment end points, limited or no follow-up, small sample sizes and the self-healing character of CL are amongst the problems encountered in therapy trials on leishmaniasis in endemic areas. In this study, a comprehensive treatment guideline is presented for returning travellers based on symptomatology, identification of the *Leishmania* species involved and the region where leishmaniasis was contracted.
Methods: Recommendations are based on literature studies and expert opinion of the staff of the Academic Medical Center, Amsterdam. Literature studies were selected if *Leishmania* infection was parasitologically confirmed and the causative *Leishmania* species was known. Studies were screened for clear clinical endpoints, clear time-points for evaluation of initial treatment success, and an adequate percentage and duration of follow-up. Both randomized controlled trials and observational studies were included. Results from studies were pooled to estimate the efficacy of specific therapies. Based on these estimations and on practical considerations of patient comfort, duration of treatment, anticipated compliance, possibility of treatment in outpatient setting, side effects and toxicity, treatment of choice and alternative treatment options were selected.
Results: Data from 167 studies were included. Based on symptomatology, infecting *Leishmania* species and country of visit, twenty five unique categories were created with 12 different *Leishmania* species. For each category a treatment of choice and alternative treatment options were defined. Treatment of choice was supported by literature evidence in 16 categories. In the other 9 categories, literature evidence was insufficient and advice on treatment was mainly based on expert opinion. Drugs advised in patients with VL included liposomal amphotericin B, miltefosine and systemic antimony. For patients with uncomplicated CL, local therapy is usually sufficient, e.g. combination therapy with intralesional antimony and cryotherapy or local antimony only. In complicated cases of CL, including MCL, systemic therapy with miltefosine, antimony or liposomal amphotericine B is advised. Therapy of choice for infections with *L. guyanensis* is pentamidine isethionate.
Conclusion: Identification of *Leishmania* species nowadays is available in most clinical cases with leishmaniasis in western countries. With varying drug sensitivities for different *Leishmania* species, therapy should preferentially be tailored to the infecting species. Our study highlights current knowledge of species-directed therapy of leishmaniasis in returning travellers, and enables an easy and fast choice of therapy to be made by clinicians. Our study also demonstrates lack of literature evidence for specific treatment of several *Leishmania* species. Well-designed and properly executed trials are needed to optimize advice on treatment in the near future.

O015

Leishmaniasis in the Netherlands 2005-2012: epidemiology, diagnostic techniques and sequence-based species typing in 192 patients

A. Bart, P.P.A.M. van Thiel, H.J.C. de Vries, C.J. Hodiamont, T. van Gool
AMC, Medical Microbiology section Parasitology, Amsterdam

Introduction: In the Netherlands, leishmaniasis is an imported disease with increasing numbers of cases. There are more than a dozen species of *Leishmania* parasites that can cause a wide spectrum of clinical manifestations, ranging from localized cutaneous leishmaniasis and disfiguring mucocutaneous leishmaniasis to potentially lethal visceral leishmaniasis. These clinical manifestations depend on both pathogen and host genetic factors, therefore species determination is of importance for prognosis and correct treatment. We here report the changing epidemiology of imported leishmaniasis in 192 patients in the Netherlands in the period 2005-2012. Moreover, we compared diagnostic techniques, and present the results of mini-exon repeat sequence typing of causative species.
Methods: Data on clinical presentation, country where leishmaniasis was acquired, diagnostic procedures and causative species were collected for 192 patients for the period 2005-2012 and stored in a database.
Results: In comparison to previous decennia, an increase in cutaneous leishmaniasis was observed. Cutaneous leishmaniasis was mainly acquired in Afghanistan, Surinam, Morocco and Spain. The majority of CL patients consisted of military personnel (56%, 102/182), partially due to an outbreak in Afghanistan. Visceral leishmaniasis and mucocutaneous leishmaniasis were rarely observed, in 8 and 2 patients, respectively.
Parasitological diagnosis was made by the combination of PCR, microscopy and culture. Compared to a combined gold standard of the three methods, sensitivities of the individual methods ranged from 73% to 98%. Microscopy was least sensitive, but is fast and cheap. Mini-exon repeat PCR combines high sensitivity and specificity, and allows differentiation between species by sequencing of the PCR product. Eight different species or species complexes were identified, allowing species-specific therapy. Four patients proved infected with *L. naiffi*, a hitherto rarely described cause of leishmaniasis.
Conclusion: In conclusion, cutaneous leishmaniasis is more frequently diagnosed in both travelers and military personnel. This calls for increased awareness among clinicians, availability of diagnostic tests and species-specific treatment guidelines in non-endemic countries.

O016

Adaptive immunity, microbiota composition and prevention of pathobiont outgrowth in zebrafish

S. Brugman[1], M. Witte[2], K. Schneeberger[1], M.R. Klein[1], B. van den Bogert[3], J. Boekhorst[4], H.M. Timmerman[5], M. Boes[1], M. Kleerebezem[6], E.E.S. Nieuwenhuis[1]
[1]Wilhelmina Children's Hospital, University Medical Center Utrecht, Pediatric Gastroenterology, Utrecht, [2]Hubrecht Institute, Utrecht, [3]Wageningen University, Laboratory of Microbiology, Wageningen, [4]NIZO food research and CMBI Radboud Nijmegen, Ede & Nijmegen,[5]NIZO food research, Ede,[6]NIZO food research & HMI Wageningen, Ede & Wageningen

Despite the great importance of understanding microbial colonization in the context of immune and metabolic disorders, it has proven difficult to dissect the innate and adaptive immune signaling pathways involved. Previously, we have set-up a model for intestinal inflammation in zebrafish. This new enterocolitis model emphasized the importance of the microbial composition in determining disease susceptibility and severity. An important feature of the zebrafish is the fact that zebrafish lack a functional adaptive immune system in the first four weeks of life. After these four weeks the adaptive immune system matures. By using this feature of zebrafish, we can evaluate effects of innate immunity on microbial composition in the absence of adaptive immunity. Our recent data suggest that T lymphocytes play an important role in regulation of the microbial composition. We observed that early during development the pathobiont *Vibrionales parahaemolyticus* is able to persist until the adaptive immune cells appear in the intestines. Upon maturation of the adaptive immune system (after four weeks) the abundance of *Vibrionales parahaemolyticus* rapidly declines. Interestingly in Rag1-deficient zebrafish (that lack adaptive immunity) Vibrionales parahaemolyticus remained abundant throughout life. Adoptive transfer of T lymphocytes, but not B lymphocytes into Rag1-deficient zebrafish dramatically reduced the presence of (pathogenic) *Vibrionales parahaemolyticus*. In conclusion, we show that T lymphocytes play a profound role in regulating the composition of the intestinal microbiota.

O018

The effect of viral, bacterial and parasitic pathogens on the intestinal microbiota

A.E. Budding, P.H.M. Savelkoul
VU University medical center, Medical Microbiology & Infection control, Amsterdam

Introduction: While investigation of the intestinal microbiota with molecular techniques has become a topic of intense research in recent years, relatively little is still known about the effect of pathogens on the intestinal microbiota. Here we analyse the effect of a number of bacterial, viral and parasitic

pathogens on the intestinal microbiota in diarrheal samples. We compare the effects of the different types of pathogens on the intestinal microbiota to each other and to a set of control samples in which no pathogen was present.

Methods: We prospectively collected 101 diarrheal samples that tested positive for gastro-enteral pathogens with conventional methods (culture, serology and PCR). Most common pathogens present were *Campylobacter* sp, *Salmonella* sp, norovirus, rotavirus and *Giardia lamblia*. Total microbiota present in these samples was analyzed by IS-pro. IS-pro is a high-throughput molecular fingerprinting method which enables a fast and automated identification of the intestinal microbiota. It is based on length variation of the 16S-23S ribosomal DNA (rDNA) interspace (IS) region combined with phylum specific sequence variation of the16S rDNA and provides relative quantification of all members of the most prominent bacterial phyla in the human intestine, including *Bacteroidetes, Firmicutes* and *Proteobacteria*. All data were analyzed with the Spotfire software package (TIBCO, Palo Alto, USA) in combination with in-house developed automated data analysis tools.

Results: In all bacterial diarrheal samples, we found relatively low abundances of the associated pathogens. Furthermore, we found a significant increase in *Escherichia coli* abundance for both bacterial and viral diarrheal samples as compared to healthy control samples. Strikingly, we found a significant increase in *Bacteroidetes* and a total disappearance of *E.coli* in all samples with *Giardia lamblia* infection. We were able to confirm this result with qPCR.

Conclusion: In conclusion, intestinal pathogens seem to have a varying effect on intestinal microbiota. Most outspoken effects seem to be in the facutatively anaerobic *E.coli* population, with a dramatic increase in bacterial and viral gastro-enteritis and a total disappearance in *G. lamblia* infection. Further investigation into the causative mechanisms behind this may provide important new insights into infectious gastro-enteritis.

O019

Streptococcus suis interaction with human intestinal epithelial cells

M.L. Ferrando[1], H. Smith[2], A. de Greeff[2], Y. Pannekoek[1], J. Stap[1], I.M.C. Vogels[1], J.A. Wagenaar[3], A. van der Ende[1], C. Schultsz[1]

[1]*AMC Amsterdam, Dept. of Medical Microbiology, Amsterdam,* [2]*Central Veterinary Institute of Wageningen UR, Dept. of Infection Biology, Lelystad,* [3]*Utrecht University, Dept. Infectious Diseases and Immunology, Utrecht*

Streptococcus suis (SS) is an important zoonotic pathogen responsible for cases of meningitis and septicemia in human patients in Asia and in the Netherlands. SS of serotype 2 (SS2) of MLST clonal complex 1 (CC1) is responsible for > 90% of reported human cases. In the Netherlands, SS2 of CC20 also contribute to human disease. In contrast, SS9, the main cause of porcine infections in northern Europe, and SS1 were never reported in human patients. The ingestion of under-cooked pork was identified as a risk factor for human SS2 infection in Asia. We hypothesized that the intestinal tract is an entry site of invasive SS2 infection and studied the host-pathogen interaction of SS with the intestinal tract, using human intestinal Caco-2-cells.

Cells were grown in 24-well tissue culture plates and on transwell filters. Differentiation to a polarized monolayer, indicated by a high trans-epithelial electrical resistance of approximately 450 Ohm, was obtained after 2-3 weeks of culture. 15 SS2 strains isolated from human patients and 8 SS2, 12 SS9, and 12 SS1 strains isolated from healthy and sick pigs, were assessed to correlate SS serotype and genotype with adhesion and translocation capacity.

All strains adhered to the Caco-2-cells, but showed low invasion capacity. Adhesion (expressed as percentage of the starting inoculum of 50 cfu/cell) increased from 15% to 34% between 1 week and 3 weeks of culture and differentiation of Caco-2-cells. Adhesion was significantly associated with serotype and genotype. The mean adherence was 27% (range 15%-44%), 12% (range 3%-27%) and 10% (range 3%-23%) for SS2, SS1 and SS9 respectively ($p = 0.0003$), at 2 weeks of cell culture. The genotypes CC1, CC20, CC13 and CC16 showed 20%, 36%, 3% and 11% adhesion, respectively ($p < 0.0001$). The invasion capacity decreased when the Caco-2-cells were fully differentiated and polarized. Invasion was 0.030% (range 0.001%-0.0574%), 0.040% (range 0.001%-0.1320%) and 0.004% (range 0.001%-0.021%) for SS2, SS1 and SS9, respectively ($p = 0.0045$), at 2 weeks of cell culture and showed a similar association with genotype as adhesion capacity ($p < 0.0001$). Assessment of translocation showed that 0.20% of the start inoculum translocated across the Caco-2-cells monolayer after 2 hours of incubation whilst the tight-junctions appeared highly damaged, as assessed by fluorescence microscopy using immunofluorescent labeled antibodies against tight-junction proteins. These results suggest that *S. suis* translocates across differentiated Caco-2-cell monolayers via the paracellular route.

Our findings show that SS adherence to differentiated Caco-2-cells is serotype and genotype specific and suggest a paracellular route for translocation across these human intestinal epithelial cells.

O020

The Staphylococcal Panton-Valentine Leukocidin targets C5a receptors

A.N. Spaan[1], T. Henry[2,3], W.J.M. van Rooijen[1], M. Perret[2,3], C. Badiou[2,3], P. Aerts[1], C.J.C. de Haas[1], K.P.M. van Kessel[1], F. Vandenesch[2,3,4], G. Lina[2,3,4], J.A.G. van Strijp[1]

[1]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht,* [2] *Universit Claude Bernard, Lyon, France,* [3] *INSERM U851, Lyon, France,* [4] *Hospices Civils de Lyon, Lyon, France*

Introduction: *Staphylococcus aureus* causes various diseases ranging from superficial skin and soft tissue infections (SSTI) to severe invasive disease. Over the last decades, community associated methicillin-resistant *S. aureus* (CA-MRSA) has become a world-wide problem. The majority of CA-MRSA strains carry the genes encoding Panton-Valentine Leukocidin (PVL). Based on epidemiological data, PVL is associated with necrotizing pneumonia and deep skin infections. PVL is a bi-component pore-forming toxin targeting neutrophils, monocytes and macrophages. Binding of LukS-PV is an indispensable trigger for LukF-PV to induce pore formation. The molecular basis of cell selectivity is not understood. The *in vitro* susceptibility of leukocytes to PVL differs considerably among mammalian species. The molecular mechanisms of species specificity have not been elucidated, hampering research into the contribution of PVL to *S. aureus* virulence.

Results: Here, we identify the human C5a receptors C5aR (CD88) and C5L2 as the receptors mediating LukS-PV binding and PVL-induced cell death. Incubation of neutrophils with LukS-PV decreases binding of antibodies directed towards the human C5aR. LukS-PV binds to cell lines transfected with C5aR and C5L2, but not to control cells. Cells transfected with these receptors are susceptible for PVL-induced pore formation, while control cells are resistant. Susceptibility of C5aR transfectants is prevented by a specific C5aR antagonist. C5L2 is expressed at low levels on neutrophils. Protection against pore formation of neutrophils by C5aR antagonists indicates that the C5aR is the major PVL receptor. Although LukS-PV binds the N-terminus of both C5aR and C5L2, the transmembrane region of these receptors is required for PVL to induce lysis. Interspecies variations of the C5a receptors drive species specificity of PVL toxicity. Cells transfected with murine C5aR cannot bind LukS-PV and are resistant to PVL-induced pore formation. In contrast however, cells transfected with rabbit C5aR bind LukS-PV comparable to that of human C5aR and are fully susceptible.

Conclusion: Taken together, identification of the human C5a receptors mediating binding and lysis of PVL explains both cell type and species specificity of PVL. These findings are of major importance for future research into PVL as a virulence factor and vaccination target.

O021

Pneumococcal meningitis: heterogenous receptor expression and the influence on *Streptococcus pneumoniae* interaction with the blood-brain barrier

F. Iovino[1], H.E. Moorlag[2], G. Molema[2], J.J.E. Bijlsma[1,3]

[1]*University Medical Center Groningen, Dept. of Medical Microbiology, Groningen,* [2]*University Medical Center Groningen, Dept. of Pathology & Medical Biology, Medical Biology section, Groningen,* [3]*Intervet International BV, MSD Animal Health Discovery & Technology – Expression, Boxmeer*

Introduction: *Streptococcus pneumoniae* (the pneumococcus) is a gram-positive human pathogen that can cause life-threatening invasive diseases such as pneumonia, bacteremia and meningitis. How *S. pneumoniae* traverses the endothelial cell layer of the blood-brain barrier is currently unclear. In this study, we address the question how *S. pneumoniae* crosses the blood-brain barrier.

Methods: Mice were intravenously infected with *S. pneumoniae*, and were sacrificed at various time points after infection to mimic the stages preceding meningitis. Bacterial localization and endothelial cells were detected on brain tissue cryostat-cut slides using immunofluorescence. Subsequently the location of various host receptors known to play a role in the interaction with *S. pneumoniae* was determined using immunofluorescence.

Results: Co-localization of *S. pneumoniae* within the vessels of the blood-brain barrier occurred at specific anatomical sites within the brain over time. Confocal analysis confirmed that *S. pneumoniae* is tightly associated with the endothelial cells. Analysis of the systemic and local immune response indicated marked differences in the local response of the brain. Analysis of the location of various known host cell receptors indicated a heterogenous expression of these receptors on endothelial cells in the brain. Currently, the consequences of this heterogeneity for the interaction with the bacteria are further investigated using IF and *in vitro* assays.

Conclusion: *S. pneumoniae* is attached to both the macro- and microvascular endothelium in the mouse brain depending on the anatomical site. The presence of bacteria in the blood elicits markedly different responses in the brain compared to the systemic response. Furthermore, there was a marked heterogeneity in the expression of known host cell receptors for *S. pneumoniae* in the brain. Ultimately, this work will lead to a better understanding of how pneumococci interact with the blood-brain barrier and cause meningitis.

O022

Staphylococcus aureus secretes extracellular adherence proteins that block neutrophil serine proteases (NSPs)

D.A.C. Stapels[1], B.V. Geisbrecht[2], K.X. Ramyar[2], F.J. Milder[1], M. Bischoff[3], M. Herrmann[3], M. von Koeckritz-Blickwede[4], K.P.M. van Kessel[1], S.H.M. Rooijakkers[1]

[1]*UMC Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*University of Missouri-Kansas City, School of Biological Sciences, Kansas City,USA,* [3]*University of Saarland Hospital,*

Institute of Medical Microbiology and Hy, Homburg / Saar, Germany, [4]University of Veterinary Medicine, Dept. of Physiological Chemistry, Hannover, Germany

The gram-positive bacterium *Staphylococcus aureus* commensally lives on human skin and mucosal tissue. However, when it becomes invasive it can cause severe infections like pneumonia and sepsis. During an infection with *S. aureus* neutrophils are indispensable. They rapidly kill bacteria via phagocytosis, contain the infection via formation of neutrophil extracellular traps (NETs), or release granular components containing antimicrobial substances. All these processes rely on the neutrophil serine proteases (NSPs): neutrophil elastase (NE), proteinase 3 (PR3), and cathepsin G (CG). Since *S. aureus* is known to evade many arms of the innate immune system, we wondered whether also the NSPs would be targeted.

By screening fractionated *S. aureus* culture supernatants, we discovered that *S. aureus* inhibits NE via the secreted extracellular adherence protein (Eap). In addition, *S. aureus* encodes two homologous proteins, EapH1 and EapH2, which also inhibit NE. With biochemical assays based on fluorescent peptide-substrates, we show that these Eap proteins also target the two other NSPs PR3 and CG, but not the less-related protease thrombin. Co-crystallography with NE and EapH1 revealed the interaction site of both molecules. As a tool for further analyses we created markerless mutants of *S. aureus* which lacked either Eap (single mutant), or all three Eap proteins (triple mutant). We compared these strains in a mouse pneumonia model. For the first time we show that the Eap homologues are virulence factors, since we recovered less bacteria from lungs infected with the triple mutant than from those infected with the single mutant. In addition, we found more neutrophils in the lungs of animals infected with the triple mutant, indicative for a role of NSPs in extravasation of neutrophils.

In conclusion, (1) this study assigns a new function to the virulence factor Eap of *S. aureus*, (2) it reports the first function of EapH1 and EapH2, and (3) it shows that EapH1 and EapH2 are important for *S. aureus* virulence. To the best of our knowledge, these are the first bacterial inhibitors of NE, CG, and PR3. This finding might eventually help to design a better treatment for *S. aureus* infections. But more importantly, it will increase our understanding of the interplay between bacteria and the immune system.

O023

Distinct localization and assembly of complement C5b-9 on gram-positive bacteria

E.T.M. Berends, F. Dekkers, R. Nijland, J.A.G. van Strijp, S.H.M. Rooijakkers
UMC Utrecht, Dept. of Medical Microbiology, Utrecht

The plasma proteins of the complement system fulfill important immune defense functions, including labeling of bacteria for phagocytosis and direct killing of gram-negative bacteria by the membrane attack complex (MAC or C5b-9). MAC is a large pore forming multi-protein complex comprised of five different proteins (C5b, C6, C7, C8, and multiple copies of C9) that incorporates into bacterial membranes. Gram-positive bacteria are considered to be resistant against MAC-mediated lysis, presumably due to their thick peptidoglycan layer. Paradoxically, several gram-positive pathogens secrete proteins that interfere with MAC formation. These findings suggest a role for the MAC in host defense against gram-positive bacteria and led us to investigate C5b-9 deposition on gram-positive bacteria.

In this study, we found that C5b-9 specifically deposits on the surface of several gram-positive bacteria during incubation with human serum. This deposition is preceded by activation of C3 and C5 since it can be blocked by specific complement inhibitors. Immunoblotting showed that bacterium-bound C9 is present in both monomeric and polymeric (SDS-stable) forms, indicating that the complex forms a tubular structure. Incubations with depleted sera revealed that these polymeric C9 structures are exclusively deposited when all C5b-9 components are present. Although MAC assembly on membranes is initiated by binding of hydrophobic C5b-7 to the lipid bilayer, on gram-positive bacteria we found that C5b mediates initial binding of C5b-9 to the surface. In conjunction, we show that C5 activation at the surface is important for C5b-9 binding, and that purified C5b-9 components cannot deposit on gram-positive bacteria. Finally, confocal microscopy reveals a surprisingly specific localization of C5b-9 on the gram-positive cell: on *S. pyogenes*, C5b-9 deposits near the division septum whereas on *B. subtilis* the complex is located at the poles. This was in contrast to C3b deposition occurring randomly on the entire bacterial surface.

In summary, our data show that (1) C5b-9 specifically deposits on gram-positive bacteria; (2) cleavage of C5 close to the bacterial surface is required for C5b-9 deposition and (3) that the complex deposits at a specific location on the gram-positive surface. These findings provide a novel view on the role of MAC in host defense against gram-positive bacteria. Importantly, this may contribute to a new paradigm of C5b-9 assembly and its physiological role.

O024

Identification of novel factors affecting *Moraxella catarrhalis* adhesion

S.P.W. de Vries, C.E. van der Gaast-de Jongh, M. Eleveld, P.W.M. Hermans, H.J. Bootsma
Radboud University Medical Centre, Pediatric Infectious Diseases, Nijmegen

Background: *Moraxella catarrhalis* is a human-restricted respiratory tract pathogen that is a common cause of childhood otitis media and exacerbations of chronic obstructive pulmonary disease in adults. During infection, *M. catarrhalis* may need to adhere to epithelial cells of different host niches such the nasopharynx during colonization and the lungs in COPD patients. Although several factors are already known to facilitate *M. catarrhalis* adherence, such as the Ubiquitous surface proteins A1/A2H, the complete repertoire of adhesins has not been completely characterized yet. In this study, we have used the genome-wide negative selection screenings technology Tn-seq to identify novel bacterial factors influencing adhesion of *M. catarrhalis* to pharyngeal and lung epithelial cells. Furthermore, we determined the transcriptional response during adherence to pharyngeal epithelial cells.

Methods: For the genomic screen, a *M. catarrhalis* BBH18 *marinerT7* transposon mutant library (~7,000 mutants) was allowed to adhere to Detroit 562 pharyngeal and A549 type II alveolar epithelial cells. After 1 hour adherence, mutant-specific probes were generated from adherent, non-adherent, and total mutant library fractions. Mutants of genes negatively selected during adherence were identified by profiling their relative abundance in the different fractions using Tn-seq (Transposon insertion site sequencing). For transcriptional profiling, *M. catarrhalis* BBH18 was allowed to bind to Detroit 562 cells and after 1 hour, RNA was isolated from the adherent and non-adherent fraction. Custom-designed Nimblegen expression arrays were used to determine expression profiles of both fractions. For validation purposes, directed gene deletion mutants were generated by allelic exchange and tested for their binding to Detroit 562 and A549 cells.

Results: Our genome-wide screen identified 11 genes as being essential for adherence to both Detroit 562 and A549 cells, and 9 genes essential for adherence to A549 only. Validation experiments using directed gene deletion mutants confirmed the role of four novel genes in *M. catarrhalis* adherence, namely aroA (3-phosphoshikimate 1-carboxyvinyltransferase), ecnAB (entericidin EcnAB), rhlB (ATP-dependent RNA helicase), and MCR_1483 encoding an outer membrane lipoprotein, with the latter mutant showing the most severe attenuation. Expression of 53 genes was increased in adherent *M. catarrhalis* BBH18 cells compared to planktonic (non-adherent) bacteria, while expression of 26 genes was found to be reduced during adherence. Among the genes with increased expression were the ABC-transporter genes for molybdate (modABC-cluster) and sulfate (cysP and cysUW) as well as genes involved in sulfate metabolism (cysD and cysH). Despite the identification of novel adhesion factors, no known adhesins were identified in our screen. Notably, neither the newly identified adherence genes nor known adhesins were differentially expressed during adhesion to Detroit 562, but rather appeared to be constitutively expressed.

Conclusion: In this study, we aimed to improve our understanding of *M. catarrhalis* adherence to respiratory tract epithelial cells by determining the genetic requirement for adherence and by monitoring the transcriptional response during contact with these cells. We identified four novel factors required for *M. catarrhalis* adherence. Finally, known and newly identified adhesins were not differentially expression upon adherence.

O025

Phosphate starvation induces the expression of a *Pseudomonas aeruginosa* ECF sigma factor and activates a virulence phenotype

K.C.J.T. Bastiaansen, A. van der Sar, W. Bitter, M.A. Llamas
VU University Medical Centre, Dept. of Molecular Microbiology, Amsterdam

Sigma factors are small proteins that associate with the RNA polymerase core (RNAPc) enzyme to direct it to specific promoter sequences, thereby initiating gene transcription. Besides a constitutively expressed primary sigma factor (σ^{70}), bacteria can encode several alternative sigma factors. The largest group of alternative sigma factors includes the so-called extracytoplasmic function (ECF) sigma factors. The activity of these alternative sigma factors is not constitutive; they are transcriptionally and/or posttranslationally activated in response to specific environmental signals. Most ECF sigma factors are co-transcribed with an anti-sigma factor. In absence of the inducing signal the anti-sigma factor sequesters the ECF sigma factor, only releasing it in response to the stimulus. A common mechanism used by gram-negative bacteria to activate ECF sigma factors is the so-called cell-surface signalling (CSS) regulatory cascade. A CSS system consists, besides the sigma and anti-sigma factors, also of an outer membrane receptor that senses the presence of an extracelullar stimulus. Most ECF sigma factors activated by a CSS mechanism respond to iron sources (i.e. siderophores, haemophores, iron-citrate) and regulate the uptake of iron into the bacterial cell.

However, our group has identified an ECF sigma factor in *Pseudomonas aeruginosa* (called σ^{VreI}) that is dedicated to the regulation of several potential virulence factors, including secreted proteins and components of secretion systems.[1] In fact, overexpression of σ^{VreI} results in increased *P. aeruginosa* virulence in the zebrafish embryo model of infection.[1] Interestingly, while expression of most *P. aeruginosa* ECF sigma factors is induced under iron depletion conditions, our recent data show that the expression of σ^{VreI} is regulated by phosphate starvation. By fusing the promoter region of the *vreI* gene to a *lacZ* cassette, we show with -galactosidase assays that this

gene is upregulated in low phosphate conditions. For this the PhoR-PhoB two component system is required, which senses and responds to phosphate starvation. Once produced, s^{VreI} mediates expression of the s^{VreI}-regulated virulence genes, which are in this way also regulated by phosphate and the PhoR-PhoB system. By direct mutagenesis we could demonstrate the presence of an active PhoB-binding site in the promoter region of the *vreI* gene. In addition, using the *P. aeruginosa* zebrafish embryo infection model we show that phosphate depletion renders *P. aeruginosa* more virulent, in agreement with previous results obtained using both a *C. elegans* and a mouse model.[2,3]. This virulent phenotype is partially due to s^{VreI} since a mutant in the *vreI* gene is nearly unable to cause mortality, even when grown under phosphate starvation conditions. This confirms that the PUMA3 system plays an essential role in the activation of virulence traits in *P. aeruginosa*.

References

1. Llamas, MA, et al. PLoS Pathog. 2009;5:e1000572.
2. Zaborin A, et al. Proc Natl Acad Sci USA. 2009;106:6327-32.
3. Zaborina O, et al. Curr Pharm Des. 2011;17:1254-60.

O026

Urinary tract infections: old and new diagnostic possibilities

N.L.A. Arents

PAMM foundation, Dept. of Medical Microbiology, Veldhoven

Objectives: Urinary tract infections (UTIs) are the most prevalent infections in primary care. Symptoms alone correlate poorly with proven infections necessitating additional diagnostic tests to confirm the presence of an UTI. Nitrite test, microscopy and dipslide culture are currently the most commonly used diagnostic tests in the primary care setting. All three tests, however, have their drawbacks. Recently, new diagnostic options have become available. Screening urine samples by flow-cytometry in order to exclude culture negative samples may be faster and more cost-effective. Direct MALDI-TOF on urine samples may offer instant bacterial determination guiding more adequate therapy. Both test, however, have not been compared directly to the current commonplace tests in the primary care setting. This study aimed to compare all available methods simultaneously in a primary care setting.

Methods: All patients consulting a single general practice (De Coevering) with symptoms suggestive for an UTI were asked to deliver an urine sample. The samples were tested at the practice by nitrite testing, microscopy, dipslide culture (Uricult trio, Orion Diagnostica) and culture on chromogenic media (Brilliance UTI Clarity agar, Oxoid). Dipslides and chromogenic media were incubated overnight at 35 °C and investigated for growth at the practice. Twice daily (11:00 and 15:00) the remainder of the samples was collected and transported to the microbiology laboratory where gram-staining, culture on colistin-aztreonam bloodagar and chromogenic media (Brilliance UTI Clarity agar, Oxoid), flowcytometry (UF1000i, Sysmex) and direct detection by mass spectrometry (MALDI-TOF, Bruker Daltonics) was performed. Gram-staining combined with culture was considered the gold standard. Proven UTI was defined as a culture showing > 10*5growth of a single species, showing > 10*5 growth of two kinds of gram-negative rods and cultures showing > 10*4 growth of a single predominant species combined with at least 3 or 4+ leucocytes in the gram-stain. Other results were considered negative.

Results: In total 500 urines were included (12% male, 88% female) of which 35% proved gold standard positive. *Escherichia coli* was the main causative micro-organism, found in 74% of the positive samples. Table 1 shows sensitivities, specificities, positive predictive values, and negative predicitive values of all evaluated tests.

Conclusion: The nitrite test seems to remain the most cost-effective screening test combining a fast result with a high positive predictive value at low cost and hands-on time. Flowcytometry and MALDI-TOF may have a niche as a follow-up test but are not suitable as a primary test.

Table 1: accuracy of diagnostic procedures for UTI in primary care

	NITRITE TEST	MICROSCOPY	DIPSLIDE	CHROMOGENIC AGAR	FLOW-CYTOMETRY	MALDI-TOF
cut-off value	nr	>20 bacteriaper field	>104 growth	>104 growth	>100 bacteria per µl	nr
sensitivity	55%	83%	92%	87%	99%	45%
specificity	99%	82%	91%	95%	39%	100%
ppv	96%	72%	86%	91%	47%	99%
npv	83%	89%	95%	93%	98%	77%

nr = not relevant; ppv = positive predictive value; npv = negative predictive value

O028

A multitarget qPCR assay for the diagnosis of bacterial vaginosis

J.W. Dorigo-Zetsma[1], E.A. Reuland[2], S. Bouter[1], P. Koenig[1], K. Gerritsen[1], L.J. Bakker[1], J.G. Kusters[3]

[1]*Tergooiziekenhuizen, Central Laboratory for Bacteriology and Serology, Hilversum/Almere,* [2]*VU University Medical Center, Medical Microbiology & Infection Control, Amsterdam,* [3]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht*

Introduction: Bacterial vaginosis (BV) is an important cause of abnormal vaginal discharge. Classically BV is diagnosed using clinical criteria (Amsel) or by laboratory based methods (Nugent score or microbiological culture). These methods suffer from observer interpretation of clinical symptoms, subjective interpretation of microscopic findings, and the inability to correctly identify the microbial flora or lack of *in vitro* growth of some relevant bacterial species. Recently culture independent molecular studies have provided more insight in the bacterial species that are associated with BV. In addition they revealed that the ratio in which different *Lactobacillus* species appear in vaginal specimen can predict the status of the vaginal flora. Here we describe the development and clinical validation of a multiplex qPCR assay for the molecular diagnosis of BV.

Methods: This prospective study was performed at Tergooiziekenhuizen, Hilversum/Almere, the Netherlands. An unselected female population consisting of 160 women = 18 years, with self-reported complaints of abnormal vaginal discharge were enrolled between April 2009-April 2010. Together with a short questionnaire, a vaginal swab for PCR and vaginal smear for microscopy was taken by their GP or gynaecologist. The multiplex real time PCR set-up targeted three BV associated bacterial species (*Atopobium vaginae, Gardnerella vaginalis, Megasphera* phylotype 1). In addition the presence of two *Lactobacillus* species (*L. crispatus* and *L. iners*) was evaluated by PCR by calculating the *Lactobacillus*-index (L-index; the ratio of the Ct-values for these two species) as an indicator of a shift to from a stable, healthy flora (both *L. crispatus* and *L. iners*) to a more pathogenic vaginal flora (mainly *L. iners*). PCR data were stratified in three groups using the classical Nugent score (NS: i.e. NS 0-3 for normal vaginal flora, NS 4-6 for intermediate vaginal flora and NS 7-10 for bacterial vaginosis). Statistical analyses were performed with SPSS, version 20.0.

Results: Data from 152/160 women were available for evaluation. The median age was 32 (range 18 to 62 years). Using NS as our gold standard 84/152 patients (55%) had normal vaginal flora, 13 (9%) intermediate vaginal flora and 55 (36%) had bacterial vaginosis. In women with BV (NS 7-10) PCR detected *G. vaginalis, A. vaginae, Megasphera* phylotype 1 in resp. 96%, 87% and 60% of the vaginal specimen, whereas in women with normal vaginal flora (NS 0-3) these bacteria were detected in resp. 26%, 6% and 2% ($p < .001$). A L-index < 1 (shift to pathogenic vaginal flora) was present in women with BV in 66%, compared to 34,5% in women with normal vaginal flora according to NS (0-3) ($p < .001$). Using NS as the reference method the BV-PCR displayed a sensitivity of 92% and a specificity of 96% with a PPV of 94% and NPV of 95%.

Conclusion: 1. Compared to the Nugent score our multiplex qPCR is a convenient and reliable tool for diagnosis of BV. 2. The Lactobacillus-index improved the correct classification of those samples in which only one of the three other bacterial species was detected.

O029

Urosepsis due to *Actinobaculum schaalii*: first case series from the Netherlands

D.W.L. Scoop, J. Gooskens, E.J. Kuijper

Leids Universitair Medisch Centrum, Medische Microbiologie, Leiden

Introduction: *Actinobaculum schaalii* is a facultative anaerobic gram-positive rod, difficult to identify by conventional biochemical tests, including commercial strips and automated identification systems. It is phylogenetically related to *Actinomyces* and is part of the commensal flora of the human genitourinary tract. In 1997, *A. schaalii* was recognized as a uropathogenic bacterium. Fewer than 50 cases of bacteremia have been reported in medical literature. In this first case series on sepsis due to *A. schaalii* in the Netherlands, we aim to describe the characteristics of these patients.

Methods: Microscopy and culture of urines were performed according to the Clinical Microbiology Procedures Handbook of ASM, including a determination of the Quality-score (Q-score) using a gram-staining of the urine sediment. Significant growth of gram-positive rods were further analyzed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Microflex; Bruker Daltonics), with confirmation by 16s rRNA sequence analysis. Since 2009, MALDI-TOF MS has been implemented in routine identification for bacteria (except for *pneumococci* and *viridans streptococci*) and yeast at Leiden University Medical Center. Blood cultures are routinely performed with BACTEC system (Becton Dickinson, Cedex, France) and bacteria are identified by MALDI-TOF MS and 16s rRNA sequence analysis.

Results: Between 2008 and 2012, we observed 4 cases of sepsis due to *A. schaalii;* two women (57 and 85 yrs of age) and two males (43 and 57 yrs of age). Three patients were hospitalized with urologic disease, of which two had infection of the higher urinary tract and one had a

catheter-associated urinary infection. In two patients the urinary tract infection was caused by a polymicrobial flora. The fourth male patient had an artificial aorta valve and developed *A. schaalii* sepsis with clinical suspicion of endocarditis. In three of four patients, urine cultures and gram-staining of the sediment could confirm the urinary tract as origin of sepsis. All four patients had functional or anatomical abnormalities of the urinary tract system. *A. schaalli* isolate from urine culture grew as small colonies and was difficult to recognize as uropathogenic bacteria without accompanying gram-staining of the sediment. All isolates were susceptible for penicillin. Patients were successfully treated and recovered completely.

Conclusion: This case series illustrates the invasive potential of the *Actinobaculum schaalii* in elderly patients with underlying urinary tract disorders. Recent improvements in identification methods facilitate the recognition of this micro-organism.

O030

Predicting outcome of urine cultures by automated urine microscopy – results of a pilot study

E. Kusters, F. Arslan, E. van Oorschot, J.C. Mercera, C.M. Cobbaert, E.J. Kuijper
LUMC, Laboratory Medicine and Clinical Chemistry, Leiden

Introduction: At the recently modernized and automated Department of Medical Microbiology (MM) of the Leiden University Medical Center (LUMC), about 60% of the urine samples offered for culture yield a negative result. Pre-examination of fresh urine samples with an automated urine analyzer has potential to reduce the number of urine cultures by over 50%, while being 99,6% sensitive in recognizing positive samples.[1] The LUMC Department of Clinical Chemistry and Laboratory Medicine (CC) recently purchased the IRIS iQ200 flow imaging microscopy urine analyzer (iQ200). Part of the prior evaluation was a pilot experiment performed jointly by CC and MM. The aim of this study was to evaluate the ability to predict a negative outcome of urine culture by a general iQ200 screen.

Methods: During a two-week pilot study 38 urine samples offered for culture were analyzed in parallel with the iQ200. Determination of the Quality-score (Q-score) using gram-staining of the sediment and subsequent urine culture was performed at MM according to Kuijper et al.[2] The Q-score represents the quality of the urine sample – possibility of contamination – and is based on the presence of squamous epithelial cells (SEC) and the ratio between SECs and leukocytes.[2]. A urine culture was considered positive if it contained at least 10^4 colony-forming units per ml, and if no more than three bacterial species were cultured.The iQ200 generates quantitative counts of elements = 3 m in diameter (erythrocytes, leukocytes, squamous epithelium, non-squamous epithelium, yeast, cylinders and rod-shaped bacteria) and of 'All Small Particles' (ASP), which are all particles < 3 m. Based on an iQ200 screen we classified urine samples with an algorithm comparable to that of Ledru, et al[3]: samples with < 5 leukocytes/L were considered negative, those with = 40 leukocytes/L positive. If samples contained 5-39 leukocytes/L and the number of ASP was < 10.000/L they were considered negative, otherwise positive.

Results: The iQ200 algorithm described above classified 17 samples as positive, among them 11 urine samples with a positive culture, while the algorithm classified the remaining 21 samples (55%) correctly as negative. Also, we found that the iQ200 was able to provide a quick alternative for the Q-score, which we called the iQ-score.

Conclusion: 1. This pilot study indicates that the iQ200 urine analyzer, which will be operational on a 24/7 basis, is able to provide a quick prescreen of urine samples offered to MM for culture that is 100% sensitive and may enable MM to reduce the number of urine cultures by over 50%. 2. An iQ200 screen may provide a 24/7 alternative for the Q-score, which is especially interesting for urine samples offered just before end of MM service hours.
Thus, the iQ200 is a platform with exchangeable information that enables a profitable collaboration between two modernized LUMC departments.

References

- Parta M, et al. Diagn Microbiol Infect Dis. 2013;75:5-8.
- Kuijper EJ, et al. Eur J Clin Microbiol Infect Dis. 2003;22:228-34.
- Ledru, et al. Paris. Ann Biol Clin. 2008;66:555-9.

O031

Large outbreak of *Salmonella* Thompson related to smoked salmon

I.H.M. Friesema[1], A.E.I. de Jong[2], D.W. Notermans[1], A. Hofhuis[1], J.H.T.C. van den Kerkhof[1], R. de Jonge[1], P.M. van Beek[1], M.E.O.C. Heck[1], W. van Pelt[1]
[1]*RIVM, CIb-Epidemiology and Surveillance, Bilthoven,* [2]*NVWA, Utrecht*

Introduction: On 15 August 2012 (week 33), the National Institute for Public Health (RIVM) noticed an increase in the number of *Salmonella* Thompson cases. Two weeks earlier, four cases had been observed and in week 33 another 11 cases were detected. Since usually around four cases of *S.* Thompson per year are seen in theNetherlands, an outbreak investigation was started.

Methods: Cases were defined as persons in the Netherlands with an *S.* Thompson cultured from any sample type, confirmed at the RIVM. A semi-structured questionnaire exploring relevant food exposures in the 7 days prior to the onset of symptoms was administered by the regional public health services, for cases with confirmation date between 15 August and 27 September. A matched case-control study was conducted by sending a similar version of the questionnaire to a random sample of persons. Four controls per case were matched on year of birth, gender, and municipality. The Dutch Food and Consumer Product Safety Authority (NVWA) performed a trace-back based upon the results of the case-control study, inspected the suspected producer and took food samples for *Salmonella* culture. Besides serotyping, pulsed field gel electrophoresis (PFGE) of the *Salmonella* isolates from patients and food samples was used in the identification of the outbreak source.

Results: Between August and December 2012, 1141 cases of *S.* Thompson were confirmed with first date of illness between 20 June and 10 November. The mean age was 43 years (range 0-95 years) with 24.7% being 18 years or younger. About one-third of the cases was male. Data on hospitalization were available for 118 cases of which 37% was admitted to the hospital. During the early weeks of the outbreak, analysis of the questionnaires yielded no clear indication as to what caused the outbreak. As the number of available questionnaires increased, in the analysis of 25 September, cases had significantly more often eaten smoked fish (45%), especially salmon, than controls (25%) (adjusted odds ratio 4.7 ($p < 0.001$)). Based upon these results, the NVWA detected *S.* Thompson in 4 of 9 sampled batches of smoked salmon. Subsequently, all smoked salmon of this producer was recalled, starting Friday 28 September (week 39). Based upon further trace-back, other products containing salmon, such as salads, were recalled during the following week. PFGE fingerprints of strains from patients and smoked salmon were indistinguishable and were different from those from other *S.* Thompson isolates not belonging to this outbreak.

Conclusion: This large outbreak of *S.* Thompson in the Netherlands was caused by contaminated smoked salmon. Based upon data from a survey performed in the Dutch population, almost half of the Dutch people eat smoked salmon at least once per month. The incriminated producer had a large market share for smoked salmon in the Netherlands, so these products potentially exposed several millions of people to the contaminated product. This explains the large number of confirmed cases and supports the statement that these cases are only the tip of the iceberg of the real size of the outbreak.

O032

Molecular epidemiology of an epidemic rise of vancomycin-resistant *Enterococcus faecium* in the Netherlands

J.C. Sinnige, J. Top, R.J.L. Willems, M.J.M. Bonten
UMC Utrecht, Microbiology, Utrecht

Introduction: The last decades *E. faecium* has emerged as one of the leading causes of nosocomial infections worldwide. These infections are difficult to treat because clinical *E. faecium* isolates have acquired resistance against numerous antibiotics, most notably against vancomycin. Except for some isolated outbreaks, infection and colonization with vancomycin-resistant *E. faecium* (VRE) was rare in theNetherlands until 2011 when several hospitals noticed an increase of VRE infections and colonization. In order to obtain insight in the molecular epidemiology of this epidemic rise, all hospital laboratories were asked to send representative isolates for molecular typing.

Methods: Representative isolates from 18 Dutch hospitals reporting an increase in VRE incidence were collected and typed using multi-locus sequence typing (MLST). PCR was used to determine the vancomycin resistance genes.

Results: In total 192 VRE (110 vanA, 81 vanB and one vanA and vanB VRE) were subjected to MLST and yielded 21 sequence types (ST); 16 different vanA VRE STs, and 9 vanB VRE STs. The most predominant types were ST117, 71 isolates in 12 hospitals and ST290, 47 isolates in 3 hospitals. The 71 ST117 isolates were represented by 51 vanB VRE (8 hospitals), 19 vanA VRE s (5 hospitals) and one vanA and vanB VRE In total six VRE clones (here defined as unique combinations of ST and van gene) were found in > 3 Dutch hospitals suggesting clonal transmission of VRE. In addition, in 5 hospitals more than three VRE clones were found, which is indicative for polyclonal acquisition. All STs belong to the three recently described *E. faecium* hospital lineages.

Conclusion: The observed increased incidence of VRE in Dutch hospitals is likely due to a combination of clonal spread and polyclonal acquisition of VRE. The latter maybe the result of horizontal transmission of vancomycin resistance genes via plasmids or other mobile genetic elements (MGE) between VRE and circulating hospital-associated vancomycin-susceptibel *E. faecium*. Further analysis of VRE clones and their vanA and vanB containing MGEs is necessary to delineate the sudden epidemic rise of VRE in Dutch hospitals.

O033

*Yersinia pseudotuberc*ulosis as cause of terminal ileitis without diarrhea in three patients

H.F. Wunderink[1], P.M. Oostvogel[2], I.H.M.E. Frénay[3], D.W. Notermans[4], A. Fruth[5], E.J. Kuijper[1]
[1]*Leiden University Medical Center, Medical microbiology, Leiden,* [2]*Medical Centre Haaglanden, Dept. of Clinical Microbiology, The Hague,* [3]*Dordrecht-Gorinchem and Albert Schweitzer Hospital, Laboratory for Medical Microbiology, Dordrecht,* [4]*National Institute for Public Health and the Environment, Laboratory for Infectious Diseases and Symptoms, Bilthoven,* [5]*Robert Koch Institute, National Reference Center for Salmonella, Wernigerode, Germany*

Introduction: The genus of *Yersinia* contains 11 species, but only *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis* are considered as human pathogens. Similar as *Y. enterocolitica*, *Y. pseudotuberculosis* is a zoonotic pathogen transmitted by the fecal-oral route through contact with infected animals or after ingestion of contaminated food or water. Main symptoms of infection with *Y. pseudotuberculosis* are enterocolitis, mesenteric lymphadenitis, or terminal ileitis.
Patients: In a period of 7 months, three unrelated cases of terminal ileitis due to *Y. pseudotuberculosis* were, diagnosed in different hospitals in the Netherlands. A previously healthy 17 year old girl and two middle-aged women without underlying diseases presented with a clinical syndrome suspected for acute appendicitis. None of the patients had diarrhea. Screening by ultrasound examination showed enlarged mesenteric lymph nodes and thickening of the terminal ileum wall. No surgery was performed. All three *Y. pseudotuberculosis* isolates were cultured from fecal samples on cefsulodin irgasan novobiocine (CIN) agar (bioMrieux Benelux B.V., Boxtel, Netherlands). Identification was performed biochemically and with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) (Bruker Daltonik GmbH, Bremen, Germany). Two of three isolates were serotyped as O1 and contained a genetically conserved 68-75 kb virulence plasmid, termed pYV, and were negative for the chromosomal ail gene of *Y. pseudotuberculosis* and for the *Y. enterocolitica* specific 16S rRNA. The third isolate was pYV and ail negative.
Conclusion: Our observation emphasizes the need to perform a fecal culture on selective media for *Yersinia* spp. when patients present with pseudoappendicitis, since *Y. enterocolitica* specific PCRs do not recognize *Y. pseudotuberculosis*. The pYV negative *Y. pseudotuberculosis* associated with terminal ileitis in this survey, is to our knowledge the second reported case of human disease caused by a pYV negative *Y. pseudotuberculosis*. We suspect underreporting of *Y. pseudotuberculosis* due the absence of diarrhea.

O034

Identification of a new biomarker for fast discrimination between epidemic *V. cholerae* O1/O139 and non-epidemic *V. cholerae* in a modified MALDI-TOF MS assay

A. Paauw[1], H. Trip[1], M. Niemcewicz[2], J.S. Olsen[3], R.E. Sellek[4], M.E.J. Heng[1], R.H. Mars-Groenendijk[1], A.L. de Jong[1], J.A. Majchrzykiewicz-Koehorst[1], E. Tsivtsivadze[1]
[1]TNO, CBRN protection, Rijswijk, [2]Military Institute of Hygiene and Epidemiology, Pulawy, Poland, [3]Norwegian Defence Research Establishment, Kjeller, Norway, [4]Ingeniería y Servicios Aeroespaciales S.A., Paseo de Pintor Rosales, Madrid, Spain

Cholera is an acute diarrheal disease caused by *Vibrio cholerae*, that can be lethal within hours if left untreated. *V. cholerae* is a category B bioterrorism agent according to the CDC. Only in 2011, a total of 589 854 cases were registered within 58 countries, including 7816 deaths. People are usually infected with *V. cholerae* by using contaminated water or food. Within *V. cholerae* there are over 200 serogroups, but only strains from serogroup O1 or O139 can cause epidemics, depending on the ability to produce the cholera enterotoxin. Other *V. cholerae* serogroups cause only occasionally a diarrheal disease which does not have epidemic potential. Rapid detection and identification of threatening micro-organisms is essential for the most effective response to an infectious disease outbreak, regardless whether the outbreak is natural or deliberate. Therefore rapid discrimination between epidemic *V. cholerae* O1/O139 isolates and all other isolates is of importance. In this study accurate discrimination between epidemic *V. cholerae* O1/O139 isolates and all other tested *V. cholerae* isolates was achieved by using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). MALDI-TOF MS is increasingly used for fast identification of (pathogenic) bacteria and has advantages over conventional techniques in being fast, cost effective, accurate and suitable for high-throughput identification. A total of 48 strains of *V. cholerae* and one of *Vibrio mimicus*, which were serotyped, screened for cholera enterotoxin genes, and genotyped using multi-locus sequence typing (MLST), were included in the study. We were able to increase the detectable range of proteins with masses up to 50 kDa by using ferulic acid as matrix in the MALDI-TOF MS assay. By comparing mass spectra, a specific marker was found that showed mass variation within 30 Da from 34,750 Da and which discriminated the epidemic *V. cholerae* O1/O139 isolates from other *V. cholerae* isolates, and even epidemic, toxin producing O1 isolates from non-epidemic O1 isolates. In all 22 epidemic *V. cholerae* O1/O139 isolates, the discriminatory peak was found, exceptin a *V. cholerae* O1 strain of the serotype Hikojima. All other epidemic *V. cholerae* isolates were from serotype Inaba or serogroup O139. Interestingly, in the spectrum of '*V. cholerae* Hikojima' another discriminatory peak at approximately 35.4 kDa was found that was absent in all other *V. cholerae* isolates. More isolates of the Hikojima serotype will have to be tested to determine whether this potential marker is strain or serotype specific. In conclusion, a fast and discriminatory MALDI-TOF MS assay was developed that discriminates between epidemic *V. cholerae* O1/O139 isolates and other *V. cholerae* isolates.

O035

Airway microbiome in Cystic Fibrosis patients: exploring culturing bias

H. Knol, J.J. Hofstra, B. de Wever, M.D. de Jong, C.E. Visser
Academic Medical Centre, Medical Microbiology, Amsterdam

Introduction: Culture-based studies of sputa of CF patients indicate that airway infection involves a relatively small collection of aerobic and anaerobic opportunistic pathogens. Many bacteria however are unculturable. Sequencing of 16S rRNA genes has revealed that microbial diversity is far more extensive. 16S rRNA sequencing is not widely available and clinicians rely mostly on culture methods. It is unknown to what extent results from cultured sputum resemble the true microbial composition in CF patients. We applied pyrosequencing of bacterial16S rRNA to compare the microbiome of unprocessed, as well as cultured CF sputum. Results were also compared to results from standard of care diagnostic protocol (SOP).
Methods: Sputum of 4 CF patients was collected and were (1) analysed using our SOP, (2) immediately underwent DNA extraction for microbiome analysis, (3) and were cultured on 2 COS agar plates (> 60 hrs) at 36 °C with 5% CO_2 and anaerobically after which all colonies were collected for microbiome analysis.
The 16S rRNA genes were amplified and pyrosequenced using 454 sequencer (Roche). Data were processed using the QIIME pipeline.
Results: On average 7215 sequences were obtained per sputum sample. Clustering sequences at 97% sequence identity, yielded 54 vs. 64 genera in directly sequenced sputum and cultured sequenced sputum respectively (19-30 vs. 24-36 per patient respectively; $p = 0.79$). Culturing significantly altered bacterial composition (UniFrac distance, $p < 0.01$). The number of unique genera found was 6 vs. 16 for directly sequenced sputum vs. cultured sequenced sputum. The results from our SOP correlated well with the most abundant genera in sequenced sputum. Culturing sputum before sequencing greatly diminished correlation with SOP results.
Conclusion: Although our SOP does not provide information on the rich bacterial diversity in CF sputum, it successfully identified the most abundant genera as assessed by direct pyrosequencing of 16S rRNA gene.

O036

Urinary antigen test established pneumococcal pneumonia has same outcome as bacteraemic pneumococcal pneumonia

S.P. van Mens[1], A.M.M. van Deursen[2], E.A.M. Sanders[2], H.E. de Melker[3], L.M. Schouls[3], A. van der Ende[4], S.C. de Greeff[3], B.J.M. Vlaminckx[5]
[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]Wilhelmina Children's Hospital, UMC Utrecht, Dept. of Pediatric Immunology and Infectious Diseases, Utrecht, [3]National Institute for Public Health and the Environment, Bilthoven, [4]Academic Medical Center, Medical Microbiology, Amsterdam, [5]St Antonius Hospital, Dept. of Medical Microbiology & Immunology, Nieuwegein

Introduction: Invasive pneumococcal pneumonia, pneumonia in which *Streptococcus pneumoniae* is isolated from blood, is regarded as a different, more severe disease entity than non-invasive pneumococcal pneumonia. It can however be questioned if there is a real clinical difference between the two. The urinary antigen test (Binax NOW) for *S. pneumoniae* has been widely accepted as a specific technique to diagnose pneumococcal pneumonia in adults. We aimed to determine the differences between non-invasive pneumococcal pneumonia established by a positive urinary antigen test and invasive (i.e. bacteraemic) pneumococcal pneumonia.
Methods: In three Dutch laboratories, where blood cultures and pneumococcal urinary antigen tests are performed routinely in the diagnostic workup of adult patients with pneumonia, all blood cultures positive with *S. pneumoniae* and all positive pneumococcal urinary antigen tests in the period from June 1, 2008 to May 31, 2010 were registered. Patient characteristics, clinical syndrome, comorbidities, disease course and outcome were retrospectively extracted from hospital records. Only adult patients (≥ 18 years of age) with a clinical diagnosis of pneumonia were included. Patients with a proven bacteraemia with *S. pneumoniae* were compared to urinary antigen test positive patients without established bacteraemia (at least one set of blood culture bottles obtained on the same day as the urinary antigen test).
Results: Of 482 patients with pneumococcal pneumonia, 249 were diagnosed by a positive blood culture and 233 by a positive urinary antigen test. Of these 233 non-bacteraemic patients in 44 (19%) only one blood culture set was obtained within two weeks before and/or after the positive urinary antigen test, in 135 (58%) patients two blood culture sets and in 54 (23%) patients at least three blood culture sets. No differences in patient characteristics were observed. The median age was 68 (standard deviation 17) in both groups. 51% and 55% of the patients were male in the urinary antigen positive group and the bacteraemic group respectively (difference non-significant by Chi-square). 75% of the patients in both groups had one or more comorbidities. The proportion of patients admitted to hospital and to an intensive care unit was equal in both groups (99% and 21% respectively). The case-fatality, defined as in-hospital death and/or death within 30 days after the first positive blood culture/urinary antigen test, was 15% in the bacteraemic patients compared to 11% in the urinary antigen test positive patients (difference non-significant by Chi-square).
Conclusion: No differences were found between adult pneumococcal pneumonia cases with a positive blood culture and cases with a positive urinary antigen test only. Both tests appear to diagnose the same disease entity when performed in the clinical setting of an adult patient with pneumonia. Invasive pneumococcal pneumonia incidence rates may be largely underestimated when only calculating blood culture positive cases.

O037

Regulation of bacterial cell wall synthesis

W. Vollmer
Newcastle University, Institute for Cell and Molecular Biosciences, The Centre for Bacterial Cell Biology, Newcastle upon Tyne, United Kingdom

Most bacteria surround their cytoplasmic membrane with a mesh-like peptidoglycan layer (sacculus) made of glycan chains that are connected by short peptides to stabilize the cell against the turgor and to maintain cell shape. In the gram-negative *Escherichia coli* the mainly single-layered sacculus locates in the periplasm and is tightly connected to the outer membrane by Braun's lipoprotein (Lpp).
Growing and dividing cells enlarge their sacculus by yet unknown molecular mechanisms.[1] According to a current model the peptidoglycan precursor lipid II is polymerized and inserted into the sacculus by multi-enzyme complexes containing peptidoglycan synthases, hydrolases and their regulators. The bi-functional class A penicillin-binding proteins (PBPs) PBP1A and PBP1B have major and semi-redundant roles in peptidoglycan synthesis, and their glycosyltransferase and transpeptidase activities have been demonstrated *in vitro*. The class B monofunctional transpeptidases PBP2 and PBP3 are essential for cell elongation and cell division, respectively. We have shown direct interactions between PBP1A and PBP2 and between PBP1B and PBP3, consistent with similar cellular localization patterns of the interacting proteins. Moreover, PBP1A and PBP2 cooperated *in vitro* in the attachment of newly synthesized peptidoglycan to sacculi.[2] Hence, our data suggest that peptidoglycan growth requires the coordinated activities of class A and class B PBPs. Dynamic membrane-attached cytoskeletal structures and associated cell morphogenesis proteins orchestrate cell elongation and division from inside the cell. In *E. coli* peptidoglycan growth is also regulated from outside the sacculus by the recently identified outer membrane lipoproteins, LpoA and LpoB, which are required for the functioning of PBP1A and PBP1B, respectively.[3] The Lpo proteins stimulate the transpeptidase activity of their cognate PBP *in vitro*. LpoB binds to the UB2H domain residing between the catalytic domains and also stimulates the glycosyltransferase activity of PBP1B. Presumably, the activation of PBPs by outer membrane lipoproteins contributes to the regulation of peptidoglycan surface density and growth rate.

References

1. Typas A, et al. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. Nature Reviews Microbiology. 2012;10:123-136.
2. Banzhaf M, et al. Cooperativity of peptidoglycan synthases active in bacterial cell elongation. Molecular Microbiology. 2012;85:179-194.
3. Typas A, et al. Regulation of peptidoglycan synthesis by outer-membrane proteins. Cell. 2010;143:1097-1109.

O038

Analysis and control of *Streptomyces* morphology in liquid-grown cultures

D. Claessen[1], M.L.C. Petrus[1], G.J. van Veluw[1], J. Gubbens[1], G.P. van Wezel[1], H.A.B. Wösten[2]
[1]*Leiden University, Dept. of Microbial Biotechnology, Leiden,* [2]*Utrecht University, Microbiology and Kluyver Centre, Utrecht*

Streptomycetes are filamentous micro-organisms that form mycelial pellets consisting of interconnected hyphae. We have recently used a flow cytometry approach designed for large particles to show that liquid-grown *Streptomyces* cultures consist of two distinct populations of pellets that differ in size. Interestingly, the size of the population of small pellets is constant, whereas the other population contains larger pellets whose diameter depends on the strain, the age of the culture and medium composition. Strikingly, deletion of the genes encoding the conserved chaplin cell surface proteins leads to disintegration of all pellets and hyphal death, inferring that these proteins play an instrumental role in pellet architecture and viability.

O039

Exogenous LPXTG containing peptides incorporation in *Staphylococcus aureus* cell wall in situ in sortase A- and growth phase dependent manner

S. Hansenova Manaskova[1], K. Nazmi[2], F.J. Bikker[2], W.J.B. van Wamel[1], E.C.I. Veerman[2]
[1]*Erasmus MC, Medical Microbiology and Infectious Diseases, Rotterdam,* [2]*Academic Centre for Dentistry Amsterdam, Dept. of Oral Biochemistry, Amsterdam*

Introduction: The majority of *S. aureus* virulence- and colonization-associated surface proteins contain an LPXTG motif that can be cleaved by a membrane-bound transpeptidase, sortase A (srtA), where after they can be incorporated into the cell wall via a covalent bond. SrtA, therefore, plays a key role in the expression of virulence factors of *S. aureus*. The aim of this study is to exploit the enzymatic properties of srtA for a novel strategy to fight *S. aureus* infections. The strategy involves development of decoy substrates containing the recognition motif for srtA (LPXTG), which will be incorporated by srtA into the peptidoglycan layer of living bacteria and can be used e.g. as a vehicle for anti-microbial compound delivery.
Methods: WT *S. aureus* (10 MSSA, 10 MRSA) and srtA KO *S. aureus* strains were exposed to fluorescently labelled synthetic srtA substrate K(FITC)LPETG-amide during growth in Luria-Bertani (LB) medium. A scrambled isomer (K(FITC)EGTLP-amide) served as a negative control. Extracellular acceptor hydroxylamide was used as an additional control for the srtA dependent specific substrate incorporation. After growth, bacteria were treated with SDS to dissociate non-covalently bound substrate. Fluorescent microscopic and FAC-scan analyses were performed to determine the incorporation. Strains with the highest specific substrate incorporation were used to study the kinetics of srtA dependent substrate incorporation in different bacterial growth phases. We quantified the srtA mRNA expression during different growth phases by qPCR in two representative strains. We synthesized K(FITC)LP**M**TG and K(FITC)**SD**LPETG (SD represents the membrane spanning domain mimic of the native fibronectin-binding protein precursor) to enhance the incorporation efficiency.
Results: *S. aureus* (WT) which was incubated with the specific K(FITC)LPETG substrate revealed highly fluorescent bacteria. The fluorescence accumulated to distinct foci at the bacterial surface. Bacteria incubated with the control substrate (K(FITC)EGTLP), as well as the srtA KO strain, revealed only a dim fluorescence. The MSSA strains showed a tendency to incorporate more srtA specific substrate than the MRSA strains ($p = 0.0753$). After SDS treatment, K(FITC)LPETG *S. aureus* (WT) still exhibited high fluorescence supporting that the specific substrate was covalently attached to the bacterial peptidoglycan layer. In line, the addition of the extracellular acceptor hydroxylamide resulted in a dose-dependent inhibition of the substrate incorporation. The K(FITC) LPETG substrate incorporation took place in a growth-phase dependent manner in a selection of WT strains. This incorporation negatively correlated with the srtA mRNA expression. The K(FITC)LP**M**TG and K(FITC)**SD**LPETG substrates variants led to a more efficient incorporation (2.5 times and 30 times, respectively in comparison to K(FITC) LPETG substrate).
Conclusion: 1. Several WT *S. aureus* strains covalently incorporate an exogenous srtA substrate in a srtA-specific and growth-phase-dependent fashion. This incorporations peaks at the stationary phase and negatively correlates with the srtA mRNA expression.
2. A significant increase of the substrate incorporation took place upon substrate modification.

O040

The role of glycosyl-hydrolases in antibiotic subsistence

T.D.J. Bello Gonzalez, H. Smidt, M.W.J. van Passel
Wageningen University, Microbiology, Wageningen

Introduction: Recently a large and diverse group of bacteria from soil, seawater, and the gut of humans and farm animals were found to not merely resist the toxic effects of antibiotics, but also to subsist on antibiotics as a sole carbon source. To date, however, no genes have been identified that enable bacteria to subsist on antibiotics, and therefore the relationship between resistance and subsistence remains unclear. We hypothesized that an intrinsic metabolic capacity to break down and utilize aminoglycosides is present in various bacteria. The aim of this study was to evaluate the role of glycosyl-hydrolases in the aminoglycoside subsistence phenotype.
Method: We tested the capacity of *Escherichia coli* DH5a, transformed with cloning vector pRSF-1b containing the neomycin phosphotransferase gene nptII, to grow on kanamycin and/or glucose in the presence of the glycosyl-hydrolase inhibiting iminosugar deoxynojirimycin (DNJ) (range of 0.00001-10 mM of DNJ). One colony of transformed cells was picked and inoculated in M9 minimal salts medium. After overnight growth, cells were washed, suspended in fresh M9 medium, and aliquots were inoculated in M9 medium supplemented with 1 mg/ml ofkanamycin and DNJ (different concentrations), and growth was followed for 24 hours at 37 °C. Glucose (1 mg/ml) was used as positive control, while M9 medium lacking any carbon source served as negative control for growth. All experiments were performed in duplicate.
Results: We found that in the presence of DNJ and glucose, the bacteria showed growth retardation, which was rapidly overcome. In contrast, DNJ halted growth completely when the only available carbon source was kanamycin, suggesting that glycosyl-hydrolases are necessary for aminoglycoside subsistence.
Conclusion: In this study we show that the presence of the glycosyl-hydrolase inhibiting iminosugar DNJ abolishes aminoglycoside subsistence. This suggests that glycosyl-hydrolase activity is required for the hydrolysis of the glycosidic bond and subsequent release of the aminosugars from the aminoglycoside. Therefore, since subsistence phenotypes on a range of antibiotics exist, it is possible that antibiotic resistance genes frequently allow not only resistance, but simultaneously facilitate antibiotic subsistence.

O041

Campylobacter jejuni induces acute enterocolitis in gnotobiotic IL-10-/- mice via Toll-like-receptor-2 and -4 signaling

M.M. Heimesaat, L.-M. Haag, A. Fischer, B. Otto, R. Plickert, A.A. Kühl, U.B. Göbel, S. Bereswill
Charité – University Medicine Berlin, Institute for Microbiology and Hygiene, Berlin, Germany

Background: *Campylobacter jejuni* is a leading cause of foodborne bacterial enterocolitis worldwide. Investigations of the immunopathology in human campylobacteriosis are hampered by a lack of suitable vertebrate models. Mice display strong colonization resistance against the pathogen due to their host specific gut microbiota composition. We have recently shown that colonization resistance can be

overcome in gnotobiotic mice as well as conventional IL-10 deficient animals with chronic colitis, but *C. jejuni* induced symptoms were rather subtle.

Methodology/principal findings: We generated gnotobiotic IL-10$^{-/-}$ mice by quintuple antibiotic treatment starting right after weaning, thereby preventing animals from commensal bacteria induced colitis. Following oral infection, *C. jejuni* B2 colonized the gastrointestinal tract of gnotobiotic IL-10$^{-/-}$ mice at high levels and induced acute enterocolitis within 7 days as indicated by bloody diarrhea and pronounced histopathological changes of the colonic mucosa. Immunopathology was further characterized by increased numbers of apoptotic cells, T- and B-lymphocytes as well as regulatory T-cells as well as elevated TNF-α, IFN-γ, and MCP-1 concentrations in the inflamed colon. Infection of gnotobiotic IL-10$^{-/-}$ mice with a commensal *E. coli* strain, however, did not induce disease indicating a *C. jejuni* specific induction of acute enterocolitis. *C. jejuni* B2 infection of gnotobiotic IL-10$^{-/-}$ mice additionally lacking Toll-like-receptor (TLR) -4 or -2 revealed that immunopathology is mediated by TLR-4- and, less distinctly, by TLR-2 dependent signalling of *C. jejuni*-LPS and -lipoprotein, respectively.

Conclusion/significance: We here present a novel murine *C. jejuni* infection model displaying acute enterocolitis and thus mimicking severe episodes of human campylobacteriosis. This acute model proves useful for further dissecting the immunopathological mechanisms underlying *Campylobacter* infections *in vivo* and to elucidate the interplay between intestinal pathogens, the commensal intestinal microbiota and the innate as well as adaptive immune system of the host.

O042

Host-pathogen interactions in Guillain-Barré syndrome

A.P. Heikema, R. Louwen, D. Horst-Kreft, B.C. Jacobs, R. Huizinga, H.P. Endtz, J.N. Samsom, W.J.B. Wamel
Erasmus MC, Medical Microbiology and Infectious Diseases, Rotterdam

It is accepted that sialylated lipooligosaccharides (LOS) expressed on the surface of *C. jejuni* activate the humoral immune system in patients with Guillain-Barr syndrome (GBS).

Host-pathogen interactions that play a role in *C. jejuni* pathogenesis include interactions that lead to enhanced invasiveness and prolonged survival of *C. jejuni* in the intestinal mucosa. We recently demonstrated that sialylated *C. jejuni* strains are more invasive in intestinal epithelial cells compared to nonsialylated strains. Epithelial invasion will likely lead to more exposure of bacterial epitopes to subepithelial immune cells, including more exposure of sialylated LOS, and therefore may contribute to the development of GBS. In addition, the surface exposed polysaccharide capsule of *C. jejuni*, associated with serum complement resistance and immune modulation, is likely involved in prolonged survival of *C. jejuni* in the host. Comparative genotyping of GBS-related *C. jejuni* strains versus strains related with uncomplicated enteritis has revealed that the capsule types HS:1, HS:2, HS:4, HS:19 and HS23/36 are dominant in GBS-related strains. These capsules may therefore be an additional virulence factor in the development of GBS.

Recognition of sialylated LOS versus nonsialylated LOS by the host immune system can be considered as an initial crucial step in anti-ganglioside antibody formation. By generating a *Campylobacter* sialyltransferase (cst-II) knockout mutant, we were able to demonstrate that sialylation of LOS enhances dendritic cell-driven B-cell proliferation through TLR4. In mice, intravenous injection of sialylated *C. jejuni* resulted in increased production of type I interferons in the spleen. Additionally, we observed that two sialic acid-recognizing immunoglobulin-like lectins (Siglecs), sialoadhesin and Siglec-7, expressed on macrophages and dendritic cells, respectively, bind to GBS-related *C. jejuni* strains in a sialic acid-dependent manner. Binding of *C. jejuni* to sialoadhesin led to enhanced phagocytosis and increased production of proinflammatory cytokines, including IL-6 and type interferons, in primary human macrophages and bone marrow-derived macrophages from mice. Such events may result in the production of anti-ganglioside antibodies as phagocytosis promotes antigen presentation whereas IL-6 and type I interferons stimulate the differentiation of B cells into immunoglobulin-secreting cells.

In summary, we conclude that interactions of *C. jejuni* sialylated LOS with the host intestinal epithelium, TLR4 and either sialoadhesin or Siglec-7 are initial events which may lead to the development of GBS. Certain *C. jejuni* capsules may additional contribute to GBS development.

O043

Dsb system of *Campylobacter jejuni* influences g-glutamyltranspeptidase activity by altering the status of the RacRS two-component system

A. van der Stel[1], A. Mourik[1], P. Laniewski[2], E.K. Jagusztyn-Krynicka[2], J.P. Putten[1], M.M.S.M. Wösten[1]
[1]*Utrecht University, Infection biology, Utrecht,* [2]*University of Warsaw, Warsaw, Poland*

Campylobacter jejuni colonize the gastrointestinal tract of birds as a commensal, however *C. jejuni* can also colonize the gastrointestinal tract of humans and other warm-blooded animals as a pathogen. Because *C. jejuni* lacks the enzyme phophofructokinase it is unable to metabolize exogenous sugars and is thus dependent on amino acids and TCA cycle intermediates as carbon- and energy source. *C. jejuni* express the enzyme γ-glutamyltranspeptidase (GGT), which has a key function in gluthatione (GSH) metabolism and contributes to persistent colonization of the mice and chicken gut. Regulation of GGT activity in prokaryotes is poorly understood. Here we report that *C. jejuni* GGT activity requires a functional disulfide bridge formation system (Dsb), although the enzyme does not contain any cysteine residues.

In silico analysis to identify a Dsb depending transcriptional unit regulating GGT revealed that the sensor domain of the transmembrane histidine kinase RacS of the two-component system RacR/RacS contains two periplasmic cysteine amino acid residues. The two-component system RacRS consists of a histidine kinase (RacS) and a cytosolic cognate response regulator (RacR) A mutation in RacR leads to has a reduced *a*bility to *c*olonies chickens indicating its importance in *C. jejuni* virulence, yet the stimulus and regulon are still to unknown.

Phosphorylation assays showed that RacS is able to autophosphorylate and consequently phosphorylate RacR, proving that the RacRS system is indeed a two-component system. RT-qPCR and electromobility shift assays showed that the *C. jejuni* GGT is activated and that RacR directly regulates expression of GGT by binding to the GGT promoter region. Western blots indicate intermolecular disulphide bridge formation between the periplasmic domain of RacS homodimers, since loss of dimers was observed in reduced RacS. Autophosphorylation assays showed that disulfide bridge formation of RacS appears crucial to keep the sensor protein in an inactive state.

Overall, our results indicate that (1) the *C. jejuni* GGT is directly regulated by the RacRS two-component system, consisting of a Histidine kinase (RacS) and a DNA binding response regulator (RacR), (2) the sensor protein RacS is a major substrate of the Dsb system and (3) disruption of the Dsb system or a Cys117Ala RacS mutation increases GGT activity. To our knowledge, RacS is the first identified Dsb substrate in *C. jejuni* and the first Dsb dependent bacterial sensor protein.

O044

A novel link between *Campylobacter jejuni* bacteriophage defence, virulence and Guillain-Barré syndrome

R.P.L. Louwen[1], D. Horst-Kreft[1], A. de Boer[2], L. van der Graaf[3], G. Knegt[1], M. Hamersma[1], A.P. Heikema[1], A.R. Timms[4], B.C. Jacobs[5], J.A. Wagenaar[3], H. Endtz[1], J. van der Oost[6], J.M. Wells[7], E.E. Nieuwenhuis[8], A. van Vliet[9], P.T. Willemsen[2], P. van Baarlen[7], A. van Belkum[10]
[1]*Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam,* [2]*Wageningen UR, Central Veterinary Institute (CVI), Lelystad,* [3] *Utrecht University, Dept. of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht,* [4]*University of Nottingham, Division of Food Sciences, Nottingham, United kingdom,* [5]*Erasmus MC, Departments of Neurology and Immunology, Rotterdam,* [6]*Wageningen University, Laboratory of Microbiology, Wageningen,* [7]*Wageningen University, Host–Microbe Interactomics, Animal Sciences, Wageningen,* [8] *Wilhelmina Children's Hospital, University Medical Center Utrecht, Depart. of Pediatric Gastroenterology, Utrecht,* [9]*Institute of Food Research, Norwich, United kingdom,* [10]*Microbiology Unit, bioMérieux, La Balme les Grottes, France*

Guillain-Barr syndrome (GBS) is a post-infectious disease in which the human peripheral nervous system is affected after infection by specific pathogenic bacteria, including *Campylobacter jejuni*. GBS is suggested to be provoked by molecular mimicry between sialylated lipooligosaccharide (LOS) structures on the cell envelope of these bacteria and ganglioside epitopes on the human peripheral nerves, resulting in autoimmune-driven nerve destruction. Earlier, the *C. jejuni* sialyltransferase (Cst-II) was found to be linked to GBS and demonstrated to be involved in the biosynthesis of the ganglioside-like LOS structures. Apart from a role in pathogenicity, we report here that Cst-II-generated ganglioside-like LOS structures confer efficient bacteriophage resistance in *C. jejuni*. By bioinformatic analysis, it is revealed that the presence of sialyltransferases in *C. jejuni* and other potential GBS-related pathogens correlated significantly with the apparent degeneration of an alternative anti-virus system: type II clusters of regularly Interspaced short palindromic repeat and associated genes (CRISPR-Cas). Molecular analysis of the *C. jejuni* CRISPR-Cas system confirmed the bioinformatic investigation. CRISPR degeneration and mutations in the cas genes cas2, cas1 and cas9 were found to correlate with Cst-II sialyltransferase presence ($p < 0.0001$). Remarkably, type II CRISPR-Cas systems are mainly found in mammalian pathogens. To study the potential involvement of this system in pathogenicity, we inactivated the type II CRISPR-Cas marker gene cas9, which effectively reduced virulence in primarily cst-II-positive *C. jejuni* isolates. Our findings indicate a novel link between viral defence, virulence and GBS in a pathogenic bacterium.

O045

Dutch contribution to malaria vaccine and novel drug development

R. Sauerwein
Radboud University Nijmegen Medical Center, Dept. of Medical Microbiology, Nijmegen

Malaria is one of the most deadly infections in the world with almost 600,000 casualties annually, predominantly in young children and mostly in Africa. In the absence of

a licensed vaccine and emerging resistance to virtually all anti-malarial drugs and pyrethroids used on insecticide impregnated bed nets, new tools are imperatively needed to combat the disease. Search for an effective vaccine and for novel drugs has been going on for decades. In some topics, academic institutions in the Netherlands play an internationally leading role with strong focus on translational research. Malaria research groups in the LUMC, BPRC, WU and RUNMC form a synergistic network with a well balanced complementary agenda from fundamental to operational research linked to an elaborate network in Africa. This Dutch research pipeline makes use of rodent, non-human primate, mosquito vector and human malaria models with specifically adapted transgenic and wild type *Plasmodium* parasites. Unique facilities required for continuous specialized parasite culture and mosquito breeding are in place using different parasites strains/species and *Anopheles* mosquitoes.
A unique tool to study malaria immunology and efficacy of immunization strategies forms controlled human malaria infections (CHMI) which has proven to be reproducible, predictable and safe for inducing *Plasmodium falciparum* (Pf) malaria. An efficient method for induction of complete protection in humans was achieved by exposing human subjects to Pf-infected mosquitoes while taking blood-stage suppressive chloroquine prophylaxis. This protocol induced 100% clinically and parasitologically sterile protection. This has opened opportunities to explore mechanisms of protective immunity, allowing the search for immune correlates/signatures of protection and clinical development of a whole sporozoite based vaccine based on (genetically) attenuated parasites.

O046

Preclinical development of genetically attenuated malaria parasites for vaccine development

S.M. Khan
LUMC Dept. of Parasitology, Leiden, on behalf of the Leiden Malaria Research Group

Introduction: Immunization with (radiation) attenuated malaria parasites (*Plasmodium*), that invade and arrest inside hepatocytes can induce sterile and long lasting protective immunity against malaria in rodent models and, importantly, in humans. Through the deletion of genes, or combination of genes, in different rodent malaria parasites, genetically attenuated parasites (GAP) have been generated. These parasites arrest during liver stage development without the occurrence of the pathogenic blood-stage parasites. Immunization with such GAPs also generates complete protective immunity in mice, even producing stronger immunity than can be achieved with irradiated sporozoites. In order to generate a human parasite, *P. falciparum*, vaccine consisting of genetically attenuated parasites, a number of critical criteria must be met, foremost safety, ensuring complete cessation of parasite development inside the liver.
Methods: Using multiple rodent malaria parasites and multiple mice strains, we describe robust and stringent screening approaches to establish safety before advancing with clinical development and studies in humans. In addition to safety criteria, we have focused on optimizing attenuated-parasite potency (determining the fewest number of parasites required for vaccination) and duration and level of protection. Advances in genetic manipulation of *Plasmodium* in conjunction with novel genomic and biological information have enabled new approaches to design genetically attenuated parasites.
Results: We have been able to create GAPs which have multiple genes deleted encoding proteins regulating independent and critical roles in parasite development in the liver, thereby creating parasites that are multiply attenuated and are unable to replicate and abort during hepatocyte development. These parasites have also been engineered to express fluorescent and bioluminescent reporter proteins permitting an examination of infection and clearance of parasites in the liver of a living mouse. These 'reporter GAP' parasites also permit an evaluation of how parasite administration (e.g. intravenously, intradermally etc) affects liver infection and therefore the induced immune response. Moreover combining the ability to both remove genes as well as introduce genes into the parasite genome has permitted us to further refine GAPs that are enhanced in their immunological potency and valency, thereby creating the next generation GAPs.
Conclusion: We have developed created GAPs that completely abort development in the liver.
Examination of GAPs in immunologically polarized mice strains has permitted us to examine and improve our immunisation protocols
Studies using reporter GAPs have permitted us to examine both GAP arrest in the liver and parasite clearance in the liver after wild type challenge of immunized animals
We have established techniques to further refine the next generation GAPs, improve their administration, and optimise the immunisation protocols as a template for human GAP immunization.

O047

Preclinical development of malaria drugs against liver stages

C. Kocken[1], A.M. Zeeman[1], S. van Amsterdam[1], E. Klooster[1], A. van den Berg[1], J.F. Franetich[2], G.J. van Gemert[3], R. Sauerwein[3], D. Mazier[2], A. Thomas[1]
[1]BPRC, Dept. of Parasitology, Rijswijk, [2]INSERM/UPMC UMR S 945, Centre Hospitalier Universitaire Pitié-Salpêtrière, Faculté de Médecine Pierre et Marie Curie, Paris, France, [3]Radboud University Nijmegen Medical Centre, Dept. of Medical Microbiology, Nijmegen

Introduction: Hypnozoites (dormant malaria liver stages) cause relapses of malaria in *Plasmodium vivax* infected people without new exposure to infected mosquitoes and are difficult to treat. Diagnostics for liver infection are not available and only primaquine provides radical cure, with risk of severe side effects especially in people with G6PD-defiiciency. To study the biology of developmental arrest, persistence and activation and to generate a screen for hypnozoiticidal drugs we have developed an *in vitro* liver stage culture system using the primate malaria *P. cynomolgi* as a model. Of all known parasites, *P. cynomolgi* has the highest genetic similarity with *P. vivax*. It has very similar biology to *P. vivax* and is one of few malaria parasites that forms hypnozoites.
Methods: Rhesus monkeys were infected with blood stage *P. cynomolgi* parasites and infected blood was used to feed *A. stephensi* mosquitoes. Sporozoites were harvested approximately 14 days after the infected blood meal and added to cultures of primary rhesus hepatocytes. Cultures were kept for 6 days, in the presence of standard antimalarials atovaquone (ATQ), primaquine (PQ) and pyrimethamine (PYR), or novel compounds to examine the drug sensitivity profile of liver stage parasites. Cultures were fixed, stained with specific antibodies and parasites were counted using automated high-content, high-throughput imaging.
Results: After *P. cynomolgi* sporozoite infection of primary rhesus hepatocyte cultures, persistent small and developing large liver forms were observed in a 10-day culture time course. The two liver stage parasite populations have differential sensitivity for ATQ, PQ and PYR, matching definitions for hypnozoites and liver schizonts.
We are testing new compounds for their *in vitro* activity against liver stages of *P. cynomolgi* parasites, and in particular to screen for activity against the small hypnozoite forms. Activity of new compounds on hypnozoite forms and/or liver schizonts has been observed in small scale screens.
Conclusion: 1. We have developed an efficient transmission platform for *P. cynomolgi*.
2. Hypnozoite-forms appear in *in vitro* cultures of *P. cynomolgi* liver stages.
3. Low-throughput drug screening has resulted in new compounds with activity against the two liver forms observed in culture.

O048

Efficacy and safety of ivermectin to prevent malaria transmission after treatment of *Plasmodium falciparum* infections with artemether-lumefantrine: a double-blind randomized controlled clinical trial

G.J.H. Bastiaens[1], A.L. Ouédraogo[2], A.B. Tiono[2], M. Guelbeogo[2], T.S. Churcher[3], R.W. Sauerwein[1], S.W. Lindsay[4], S.B. Sirima[2], C. Drakeley[5], T. Bousema[1,5]
[1]Radboud University Nijmegen Medical Centre, Dept. of Medical Microbiology, Nijmegen, [2]Centre National de Recherche et de Formation sur le Paludisme, Ministère de la Santé, Ouagadougou, Burkina Faso, [3]Imperial College London, Dept. of Inf. Disease Epidemiology, London, United Kingdom, [4]Durham University, School of Biological & Biomedical Sciences, Durham, United Kingdom, [5]London School of Hygiene and Tropical Medicine, Dept. of Immunology & Infection, London, United Kingdom

Introduction: Resistance of malaria parasites against artemisinin-based combination therapies (ACTs) has emerged in Southeast Asia and is threatening malaria control efforts worldwide. Novel strategies to prevent the spread of artemisinin-resistant malaria infections are urgently needed. One of these novel strategies may comprise a combination of ACT with ivermectin (IVM). IVM reduces the lifespan of malaria-transmitting mosquitoes after feeding on humans treated with IVM and could form part of a drug combination that not only treats malaria patients but also prevents post-treatment malaria transmission. We determined the impact of a single or double dose of IVM in combination with artemether-lumefantrine (AL) in reducing malaria transmission.
Methods: We determined the duration of the mosquitocidal effect of IVM in Swiss mice, Wistar rats and Cynomolgus monkeys. Blood samples from IVM treated animals were offered to *Anopheles stephensi* mosquitoes by direct skin feeding. These outcomes informed a clinical trial that has currently started enrolment. In a double-blind randomized controlled clinical trial we are enrolling 120 healthy volunteers (15-25 years) who are asymptomatically infected with *P. falciparum* parasites. Forty participants receive AL alone, 40 receive 200 g/kg IVM with the first dose of AL, and 40 receive 200 g/kg IVM with the first and the fifth dose of AL. Blood samples are offered to *An. gambiae s.s.* and *An. funestus* mosquitoes using an experimental membrane feeder set-up on days 1, 3 and 7 after initiation of treatment.
Results: The animal studies indicated that mosquito mortality was 70-100% when mosquitoes fed 1-2 days after the last IVM administration. After this time-point the mosquitocidal effect is still evident in some animals but becomes more variable. Results of the clinical trial will be available by the end of March 2013 and presented to describe the safety of the combination of IVM and AL and the proportion of (malaria infected) mosquitoes surviving sufficiently long to complete the sporogonic cycle of *P. falciparum*.
Conclusion: Our finding of a pronounced but short-lived mosquitocidal effect has implications for the usefulness of IVM as an additional intervention to prevent the spread of resistance by blocking transmission shortly after ACT treatment.

O049

Complete protection against malaria after sporozoite immunization of volunteers under chloroquine prophylaxis is mediated by pre-erythrocytic immunity

E.M. Bijker[1], G.J.H. Bastiaens[1], A.C. Teirlinck[1], G.J. van Gemert[1], W. Graumans[1], M. van de Vegte-Bolmer[1], R. Siebelink-Stoter[1], T. Arens[1], K. Teelen[1], A. Jansens[1], D. Zimmerman[1], M. Vos[1], B. van Schaijk[1], J. Wiersma[1], A.J.A.M. van der Ven[2], Q. de Mast[2], L. van Lieshout[3], J.J. Verweij[3], C.C. Hermsen[1], A. Scholzen[1], R.W. Sauerwein[1]

[1]Radboud University Nijmegen Medical Centre, Dept. of Medical Microbiology, Nijmegen, [2]Radboud University Nijmegen Medical Centre, Dept. of General Internal Medicine, Nijmegen, [3]Leiden University Medical Center, Dept. of Parasitology, Leiden

Abstract not yet approved for publication.

O050

HRMO and other infectious diseases outbreaks in nursing homes

W.P. Achterberg

LUMC, Dept. of Public Health and Primary Care, Leiden

Nursing home residents are at great risk for infectious diseases. This is caused by host risk factors, as well as risks inherent to institutional living. Host risk factors include ageassociated changes in immunity (e.g., B- and T-cell function) and may lead to reduced responsiveness to vaccinations (e.g., influenza, *S. pneumoniae* vaccines) and to increased susceptibility to systemic infections and reactivation of latent infections (e.g., *Mycobacteria,* and varicella-zoster virus). Additionally, multiple comorbidity (e.g., diabetes mellitus, COPD, impaired dentition) place nursing home residents at increased risk of common community-acquired infections including pneumonia, UTI and wound infections. Institutional living within a nursing home unit promotes common source respiratory (influenza, Legionella) and gastrointestinal (norovirus) outbreaks.Treatment with antibiotics is often empiric which places residents at increased risk of subsequent infection with antibiotic-resistant pathogens (e.g., methicillin-resistant *Staphylococcus aureus,* vancomycin-resistant *Enterococcus* species, multidrug-resistant gram-negative bacilli) and *Clostridium difficile*. These antibiotic resistant pathogens can subsequently be secondarily transmitted to uninfected residents of the same nursing home, as well as to hospitalized patients when nursing home residents are admitted for non-infection related acute illness. Nursing home professionals colonized with resistant pathogens can also contribute to colonization of residents because of intense and prolonged contact with residents. Therefore, nursing home residents can serve as unintentional vectors that shuttle clinically relevant pathogens from the nursing home to the hospital, and back to the nursing home, and impact on the infectious disease burden of an entire community. Given the recently transition of policy to more home care, the demographics of nursing home residents have shifted to the oldest adults with even more extensive comorbidity and functional disabilities who are most vulnerable to infection.

The serious consequences of antibiotic resistance in long-term care facilities provide a rationale for the conduction of research and the development of policies directed at reducing antibiotic resistance in these facilities. These should focus on both the implementation of infection control measures and antibiotic stewardship. With regard to infection control measures, training of health care personnel is crucial to implement hygiene practice. To establish a sustainable training program, facilities should allocate adequate resources. The assignment of an infection control committee or an infection control practitioner may facilitate the development and sustainability of such a program. With regard to antibiotic stewardship, it is important that physicians are well educated on the diagnosis and treatment of infectious diseases in residents, and that this education is based on relevant guidelines. Other measures to facilitate appropriate antibiotic prescribing include monitoring of antibiotic use, encouraging physicians and pharmacists to develop and regularly review formularies, and promoting specimen culturing in residents with suspected infection.

O051

HRMO – what can you expect from Municipal Health Services?

A.J.M. Schreijer[1], A.M.L. Tjon-A-Tsien[2]

[1]Municipal Health Service Utrecht, Dept. of communicable disease control, Utrecht, [2]Municipal Health Service Rotterdam Rijnmond, Dept. of Control of Infectious Diseases, Rotterdam

Introduction: The burden of highly resistant micro-organisms (HRMO) is increasing, including higher mortality and longer hospital stays than disease caused by susceptible strains and consequently increasing healthcare costs and productivity losses. These aspects make HRMO a public health threat. The principal task of the Municipal Health Service (MHS) is public health protection, including infectious disease control. Therefore, the MHS is a key player in the fight against HRMO during and between outbreaks.

Role of the Municipal Health Service: The MHS generally informs citizens about prudent use of available antibiotics and promotes effective hygienic behaviour at home and abroad, thus adding to antimicrobial stewardship. Through the MHS position in regional surveillance infectious disease networks, the MHS can help elucidate novel epidemiological pathways in the community and act as a binding factor between institutions. During outbreaks in hospitals and nursing homes the MHS can play a complementary role, including the following tasks disentangled from previous outbreaks:

1. The MHS carries out action in the region to prevent and control the spread of HRMO outside the hospital.
2. The MHS can coordinate the process of developing regional policy with several chain partners such as nursing homes, home care organisations and GP's.
3. The MHS can provide advice on (risk) communication with citizens and patients.
4. The MHS can make sure that local authorities are timely informed.
5. When outbreaks reach the level of a disaster (e.g. great disturbance among the population or hospitals cannot handle the amount of patient anymore) the MHS is a key player in disaster management on a regional level.

The involvement of the MHS differs per situation, HRMO, and institution. Inside hospitals, the knowledge and expertise of fighting HRMO is high among their professionals (medical microbiologists and advisors infection prevention). In this situation the MHS can offer tailored advice, depending on the need. In other situations, such as an outbreak in a home care setting, the MHS will have a more prominent role.

Discussion: Unfortunately, outbreaks of HRMO in institutions are not always reported to the MHS. A possible explanation is that the complementary role of the MHS is unknown or that involvement of the MHS is sometimes not appreciated. However, if the MHS is not aware of an outbreak, it cannot perform its complementary and epidemiological tasks. The National Institute for Public Health and the Environment (RIVM) is now, together with several partners including the MHS, working on a national guideline for HRMO in the public health setting. This guideline will give professionals more grip on dealing with HRMO outside hospitals. However, a lot of the do's and don'ts in the control of HRMO in the public health setting remain unknown. Hence the need for hospitals, nursing homes and MHS to work together on research into effective strategies in the control of HRMO in a public health setting is a strong one.

Conclusion: Inform and invite the communicable disease control professionals at your MHS so they can work together with you on tackling the HRMO problem.

O052

Holistic risk assessment identifies large variation in infection control practices and outcome in Dutch nursing homes

L.E. Willemsen[1], J. Nelson-Melching[2], Y. Hendriks[1], A. Mulders[3], S. Verhoeff[3]

[1]Amphia hospital, Laboratory for Microbiology and Infectioncontrol, Breda, [2]Sint Elisabeth Hospital, Tilburg, [3]THEBE

To identify infection risks we developed a new method with a holistic approach that gives insight in the overall infection risks in health care settings. This infection control risk model includes outcome variables as well as patient- and, ward-related variables, which are visualised in a, easy-to-read, plot. This abstract describes the design, applicability and results of the infection risk plot performed in Dutch Nursing Homes (NH).

The model can be filled with variables depending on the setting and population. We included the following 7 variables: use of medical devices, prevalence of health care associated infections (HAI), antimicrobial therapy, rectal carriage of extended spectrum bèta-lactamase (ESBL) producing bacteria, environmental contamination, availability of local guidelines , shortcomings in infection prevention preconditions. Furthermore the characteristics of the patient population were determined. All variables were categorised as low risk, medium risk and high risk, based on literature or expert opinion and presented in an infection risk plot, overall as well as per NH.

A total of 774 residents were included in 9 NH. No differences were found in the characteristics of the patient population between the different NH. Large differences were found in the other variables with a distribution across all 3 risk categories. This resulted in different risk-plots for the different NH. Most striking differences were observed in the prevalence of ESBL carriage between the different NH ($p < 0.001$). Multivariate analysis showed that ESBL carriage was associated with shortcomings in infection prevention preconditions ($p < 0.001$). Micro-array and AFLP results suggest there were clusters of ESBL positive *E.coli* within a couple of NH. One large cluster of one cloon, found in 5 NH, included 3 different ESBL genes (TEM, CTX-M1-15 and CTX-M9). MLST typing is in progress.

The infection risk plots demonstrated substantial differentiation. Problem areas were shown at a glance. We found a large variation in infection control practices and prevalence of ESBL carriage between different NH. Based on these results a tailor made, targeted quality improvement project can be executed and the results can be measured in a repeated measurement. This makes this tool an important aid for quality improvement in NH.

O053

National surveillance of carbapenemase producing *Enterobacteriaceae* in the Netherlands 2011-2012

D.W. Notermans, K. van der Zwaluw, A. Haenen, J. Alblas, J. Muilwijk, S. De Greeff, L. Schouls, M. Heck, H. Bijlmer

RIVM, CIb, Bilthoven

Introduction: Carbapenemase producing *Enterobacteriaceae* (CPE) are an important threat to health care. Besides resistance to the class of bèta-lactam antibiotics, isolates often carry genes associated with resistance to other classes of antibiotics, leaving limited possibilities for antibiotic therapy for infections with these strains. To assess the extent of the problem of CPE in the Netherlands, a national surveillance was started.

Methods: As of 2011, the national surveillance of CPEis performed by the RIVM. Medical microbiological laboratories are asked to submit all *Enterobacteriaceae* with a minimum inhibitory concentration (MIC) for meropenem > 0.25 or imipenem > 1 mg/L for phenotypical and genotypical confirmation of carbapenemases. The presence of carbapenemase genes is assessed by PCR for KPC, NDM, OXA-48, VIM, IMP, GIM, SIM, and SPM. Meropenem MIC is determined by Etest. Further strain- and epidemiological information is collected by web based questionnaire. In 2011, a large outbreak in one hospital with an OXA-48 positive *Klebsiella pneumoniae* occurred and isolates obtained from this hospital during the outbreak were excluded from this description of the surveillance results.

Results: In the two year period, 438 strains from 379 patients were submitted, yielding 91 CPE isolates from 82 patients (36 in 2011 and 55 in 2012). From 7 patients, two different *Enterobacteriaceae* species were isolated that harboured the same carbapenemase gene. From one patients, two different species were isolated each carrying a different carbapenemase gene and from one patient two different carbapenemase genes were found in the same species.

Carbapenemase genes found were OXA-48 (54x), NDM (15x), KPC (14x), VIM (4x) and IMP(4x).

Species involved were *K. pneumoniae* (67x), *Escherichia coli* (12x), *Enterobacter cloacae* (9x) and other (3x). 40 cases concerned a *K. pneumoniae* with OXA-48 (22 from 2011 and 18 from 2012).

Meropenem MICs were within sensitive range (≤ 2 mg/L) in 37 and resistant (> 8 mg/L) in 32 isolates. Isolates with an OXA-48 were relatively more frequently within sensitive range (56 vs. 40%).

Of 55 patients for whom epidemiological information was provided, 43 had been abroad, 24 of whom to North Africa or the Middle East. For 28 a hospitalisation abroad less than 6 months ago was reported. For 5 patients, all from 2011, a connection to the Dutch outbreak hospital was reported.

Conclusion: Apart from the large OXA-48 outbreak in 2011, no other large CPE outbreaks were detected during the surveillance and only a limited number of CPE were seen in the Netherlands. The surveillance is on voluntary base and it is therefore likely not have complete national coverage yet. The number of strains submitted and the number of CPE isolates found increased from 2011 to 2012, which may reflect improved participation during the second year of the surveillance.

K. pneumonia was the predominant species (73%) and OXA-48 the most frequently encountered (59%) carbapenemase, but this was lower in 2012 than in 2011.

As far as known, the majority of cases could either be related to admissions in a foreign hospital or to the Dutch outbreak hospital.

O055

Human papillomavirus (HPV) dynamics in the Netherlands

M.A.B. van der Sande[1], H. Boot[1], F. van der Klis[1], A. King[1], M.F. Schim van der Loeff[2], H. Boogaards[1], H.E. de Melker[1]
[1]*RIVM, CIb/EPI, Bilthoven,* [2]*Public Health Services, Infectious Disease Research, Amsterdam*

Human papillomavirus (HPV) infections are the most common STI, with prevalence increasing rapidly following sexual debut. Infection with high-risk HPV types is a leading cause of anogenital cancers and a subset of oro-pharyngeal cancers while HPV vaccination can reduce HPV related (pre)malignancies. As vaccination is most effective prior to acquiring HPV infection optimal time of vaccination is prior to sexual debut. The discussion is ongoing to what extent vaccination at a later stage in life could also be (cost)effective. In theNetherlands, vaccination was introduced in 2010 for girls aged 12 years aiming to reduce cervical cancer cases. A one-off catch-campaign was performed in 2009 for girls born in 1994-1996. Two vaccines, a bivalent HPV16/18 vaccine (cervarix) and a quadrivalent HPV6/11/16/18 (gardasil) for the prevention of HPV infections are registered. At present based on European tendering procedure the bivalent vaccine is used. In some other countries boys are targeted for vaccination as well and/or catch up campaigns for sexually active people are offered up to the age of 26, or the quadrivalent vaccine has been used that also prevents genital warts.

In the Netherlands, vaccination of girls is likely to change the HPV dynamics in females and (heterosexual) males. The expected long-term impact is a reduction in the incidence of HPV related (pre)malignancies, but there can also be a major impact on the interaction between HPV and other STI, including HIV. To improve our understanding of HPV dynamics, including early assessment of the impact of vaccination prior to assessment of HPV related (pre) malignancy from 30 years onwards (start cervical cancer screening), several studies are ongoing in the Netherlands, next to studies focusing on vaccine uptake, vaccine safety, reactogenicity and clinical outcomes.

These HPV dynamics studies are not limited to the population currently targeted for vaccination, and include:

a) Seroprevalence studies in the general and in high-risk populations (PIENTER studies).

b) Cohort study in girls recruited in 2009 at age 14-15 years prior to start of vaccination, including both vaccinated and unvaccinated girls, with annual follow up of vaginal virology, vaginal immunology, serology and epidemiology (HAVANA).

c) Cohort study among sexually active women 16-29 years old who participated in at least two annual rounds of a *Chlamydia* screening programme (CSI-HPV).

d) Cohort study among men who have sex with men, with and without HIV-infection, with 6-monthly follow up of epidemiology, serology, and virology of anal, penile and oral samples (H2M).

e) Biannual cross sectional survey among 16-24 year old visitors of STI centres, analysing HPV virology and serology in relation to other STI (PASSYON).

f) Mathematical modelling, sero-informatica and cost-effectiveness analysis.

The first results of population-based and of (high-risk) targetted populations studies show considerable variation in pre-vaccination (sero)prevalence according to age and population; e.g. the prevalence of high-risk HPV was 2.7% in girls in the general population (HAVANA) and 58% in STI visitors.

A brief overview will be given of the first results, and potential implications of these studies will be discussed.

O056

The dynamics of gonorrhoea in the Netherlands

J.E.A.M. van Bergen[1,2]
[1]*University of Amsterdam-AMC, Dept. of General Practice, Amsterdam,* [2]*RIVM-National Centre for Public Health and the environment, Bilthoven*

In 2011 3,575 patients were diagnosed with gonorrhoea in the STI centres in the Netherlands. More than half of the infections occurred among Men who have Sex with Men (MSM). The positivity rate among MSM was 9%, 5-fold that of heterosexuals. Most infections among MSM are anorectal infections. The number of oral infections increased significantly in the past 5 years, especially due to increased oral testing. Among heterosexual visitors of STI clinics the positivity rate is two-fold higher among visitors originating from Suriname, Antillen and/or Aruba compared to Netherlands residents. Trend analysis shows relative stable positivity rates during the last 5 years among MSM and heterosexual persons visiting STI clinics. Co-infections occur regularly, especially co-infections with Chlamydia trachomatis (29%).

General practitioners are the core provider of primary care in the Netherlands. It is estimated that general practitioners (GP) provide 2/3 up to 3/4 of the STI care in the Netherlands. However, the estimated number of gonoccocal infections in primary care is less than at STI clinics (sentinel surveillance data of GP (LINH): 2,588; 95% CI 2,297-4,544). More high risk groups are captured at STI centres.

Persons visiting STI clinics, or persons visiting GP are not representative for the general population. The true population prevalence of gonorrhoea is unknown. Unlike Chlamydia, gonorrhoea is much more concentrated among high-risk groups. A survey in 2005 aimed at establishing population-based data on Chlamydia prevalence showed that (asymptomatic) Chlamydia infections were prevalent among 2.1% of the general population aged 15-29 years. However in a nested study, no gonococcal infections were found in a subset of 600 CT negatives and only 4 infections in a subset of CT-positives, who all had either symptoms or other risk factors. Data from a recent study, exploring gonorrhoeal positivity among those participating in a systematic Chlamydia screening pilot, will be presented.

Resistance is a growing problem in gonorrhoeal treatment. The bacteria became resistant to sulfonamides in the 1940s, then to penicillins and tetracyclines in the 1970s and 1980s. Fluorquinolones became in turn of the century the medicine of choice, but resistance developed very quick and rose from 7% in 2002 to 27% in 2005, and in recent years up to 30-50%, making ciprofloxacine not the drug of choice any more, and 3rd generation cephalosporin were advocated, in particular ceftriaxone 500 mgr IM. Although new guidelines at STI clinics were implemented quickly, prompt adoption and adherence to in general practice is a problem, since guidelines are not frequently adapted and individual GP do not have much experience with gonococcal infections. A study on prescription patterns of Dutch GP showed that it took up to 5 years before more than 60% prescribed cephalosporin. Still in 1/4 ciprofloxacine is used, although it is not known if this occurs as initial treatment or after culture and resistance assessment. Last year several papers warned for multi-resistant strains against current antibiotics. In the Netherlands resistance patterns surveillance is implemented via GRAS. Although some reduced susceptibility for cefaxim is found, up to now no treatment failure is reported in the Netherlands. Treatment options for cephalosporin-resistant gonorrhoea are very limited. Adoption of any new treatment options and/or changing guidelines presents a challenge, especially in primary care. Clinical support systems in the EPD provide new and innovative avenues for quality improvement. Reinvestment in gonorrhoea prevention, diagnostics and control and research into new treatment options are urgently needed.

O057

False-positive *Neisseria gonorrhoeae* results in urine samples using a highly sensitive NAAT tests, resulting in a pseudo-outbreak of gonorrhoea

A.P. van Dam[1], K. Adam[2], S.M. Bruisten[2], A.G. Speksnijder[2], P. van Leeuwen[2], H.J. de Vries[2]
[1]*OLVG, Medical Microbiology, Amsterdam,* [2]*Amsterdam Health Service, Public Health Laboratory, Amsterdam*

Urines from male patients visiting our STD outpatient department with a low risk for STD are routinely tested by NAAT (Aptima Tigris Combo 2). Between August 17th and September 21st, 2012, 62 (7.3 %) of these patients showed a positive result in urine tested in the NAAT for *Neisseria gonorrhoeae* (NG). This was in marked contrast to the low frequency (0.8%) of positive results between January 1st and August 16th among such patients. During this outbreak period, the prevalence of NG among high-risk patients as determined by culturing, and of positive NG NAAT results in urines received from other practices and from vaginal, rectal and tonsillar swabs from the STD department remained unchanged. The prevalence of positive NAAT results for *Chlamydia trachomatis* (CT) in urines from low-risk male patients was only 3.9% in the outbreak period, compared to 5.9% in the earlier part of the year. This suggested a laboratory artefact rather than a true STD outbreak and prompted us to look for the causes of this increase. A confirmatory NG culture was positive in only 2/24 patients from whom cultures were obtained. A confirmatory NG DNA-PCR was positive in the two patients who had a positive culture; in addition, a very weak signal (Ct value > 40), routinely considered to be negative, was found in 9/58 of the probably contaminated samples.
All 5 swabs taken from various locations in the male bathroom, as well as all 4 swabs taken from transport trays for samples were positive in the GC NAAT; only 1 of these 9 swabs was additionally positive in the CT NAAT. In contrast, swabs taken from the trays used in the testing area only were negative; these trays had routinely been cleaned with chlorine. The NG DNA-PCR was negative for all environmental samples. An audit showed that some clients do not deliver their urine samples in a hygienic way, administrative employees who transferred urine into Aptima tubes did not strictly follow the guideline of not touching the seal of these tubes and the procedure of pipetting urines in Aptima tubes showed shortages.
Daily cleaning of bathroom facilities and trays with chlorine (instead of previous daily cleansing with regular ionic detergents), in combination with strictly following adjusted anti-contamination guidelines led to an end of this pseudo-outbreak. Thirty-seven patients, to whom the positive NG test result had already been reported, were informed of the doubts of their gonorrhoea diagnosis. During the remainder of the year 2012, only 0.2% of low-risk male patients had a positive GC NAAT result in the urine.
Most likely, this pseudo-outbreak was a consequence of a combination of external contamination of trays and test tubes with NG-RNA, in combination with inadequate handling of tubes at the laboratory. False positive STI results and pseudo-outbreaks are conceivable in situations where self-collection of samples for NAAT tests is performed by patients. In these situations frequently cleaning of collection sites with nucleic acid degrading solutions like chlorine, in combination with strict processing procedures in the laboratory, is strongly advisable.

O058

Low prevalence after systematic screening for *Trichomonas vaginalis* in three patient cohorts from general practitioners, STI clinic and a national population based *Chlamydia* screening study

T.H.B. Geelen, C.J.P.A. Hoebe, J.C.A.M. Dirks, N.H.T.M. Dukers, P.F.G. Wolffs
Maastricht University Medical Center, Medical Microbiology, Maastricht

Introduction: Among sexually transmitted infections (STIs), *Trichomonas vaginalis* (TV) is the most common non-viral STI worldwide. However, TV is an under-recognized and under-diagnosed infection while it could facilitate HIV-transmission. The introduction of polymerase chain reaction (PCR) tests allows for high-throughput sensitive detection of TV. Yet only a limited number of studies focused on TV prevalence before, especially a limited number of European studies. Therefore a multi-cohort study was performed to investigate the prevalence of TV among three distinct Dutch patient populations and its relation with *Chlamydia trachomatis* (CT) co-infection.
Methods: A total of 2089 participants, men and women, were included between 2008 and 2012 from three distinct cohorts. A total of 812 participants from the population-based national *Chlamydia* Screening Intervention (CSI) study, 675 attendees of the STI clinic south Limburg and 602 patients from general practitioners (GPs) were included. From the CSI study and the STI clinics, a supplement of CT positives was included to assess TV and CT co-infection. All participants were systematically assessed for TV using Taqman real-time PCR analysis. Presence of CT was assessed with the Cobas 4800 for all samples from the STI clinics and GPs. Samples from CSI were previously tested for CT within local laboratories by three different PCR-assays (BD Probetec, Genprobe Tigris, and Roche Cobas Taqman PCR). Associations between TV and CT co-infection and age were studied by dividing patients' age in 4 age categories (i.e. < 21 years, 21-30 years, 31-40 years and > 40 years old).
Results: The overall prevalence of TV was 0.9% among the three distinct cohorts. The highest TV prevalence was observed in the GP cohort (1.5%), however no significant differences between the 3 study populations were observed (STI clinic (0.6%) and population-based cohort (0.7%)). TV was found in 0.7% of the CT positives and a similar 1.1% among CT negatives. The CT prevalence decreased with increasing age, and was highest at an age < 21 years (15%; $p < 0.05$). In contrast, TV prevalence was highest in the age group > 40 years (2%), but did not differ significantly from other age-groups.
Conclusion: The prevalence of TV was low (< 2%) and similar between all three populations studied. We found no association between TV and CT.

O060

Paramyxoviruses crossing the species barrier: a continuing story

A.D.M.E. Osterhaus
Erasmus MC, Viroscience, Rotterdam

The family *Paramyxoviridae* is a group of enveloped viruses that contain an unsegmented single stranded RNA genome of negative polarity. Paramyxoviruses transmit from host-to-host via the respiratory route and are responsible for significant diseases in humans and animals. The family *Paramyxoviridae* contains two subfamilies, the *Paramyxovirinae* and *Pneumovirinae*. The subfamily *Paramyxovirinae* contains the genera morbillivirus (including measles virus), rubulavirus (including mumps virus), respirovirus (including the parainfluenzaviruses) and henipahvirus (including hendra and nipah virus). The genus avulavirus includes Newcastle disease virus, which is an important pathogen of birds. The subfamily *Pneumovirinae* contains the genera pneumovirus (including human respiratory syncytial virus) and metapneumovirus (including human metapneumovirus). The majority of the paramyxoviruses are restricted to a single or a limited number of host species. Interestingly, the phylogenetic relationships of these viruses often parallel those of their respective host species. However, this did not result from co-evolution between the viruses and their hosts, as the evolutionary diversification of mammals is much older than that of the paramyxoviruses. Rather, these viruses have evolved from common ancestral viruses that have adapted to their respective mammalian hosts. Thus, paramyxoviruses causing disease in humans generally have a zoonotic origin. In some cases this origin is thought to be relatively recent: HMPV is closely related to avian metapneumovirus or turkey rhinotracheitis virus and is though to have jumped species between 150 and 200 years ago. The virus was first identified as a major pathogen of humans in 2001, but on basis of archival sera we were able to demonstrate the prevalence of HMPV in the early 1960s. Measles virus is thought to have evolved from a common morbillivirus ancestor when humans started to live in large communities in close proximity to livestock harbouring rinderpest-like viruses. Interestingly, morbilliviruses have been identified as the causative agent of several large disease outbreaks in marine mammals. In most cases the outbreaks were related to cross-species transmission events from terrestrial or marine mammals. In recent years canine distemper virus was identified as the causative agent of disease outbreaks in captive non-human primates, demonstrating that this morbillivirus can cross the species barrier from carnivores to primates. This may have important consequences if measles virus were to be eradicated by vaccination. More recent examples of zoonotic paramyxovirus transmission in humans can be found in the henipah viruses: hendra and nipah virus originate from bats and cause lethal outbreaks among humans following infection of intermediate hosts such as horses or pigs, respectively. In this respect, the recent identification of a plethora of undescribed paramyxoviruses in different bat species suggests that similar zoonotic transmission of paramyxoviruses to humans may occur more frequently than currently thought.

O061

Mapping the molecular events during adaptation of canine distemper virus to primates

W.P. Duprex
Boston University School of Medicine, Dept. of Microbiology and National Emerging Infectious Diseases Laboratories (NEIDL), Boston, USA

Eradicating a virus by sustained vaccination is a tremendous achievement and illustrates how delivery of these complex biopharmaceuticals has had a huge impact on human and animal health. However, it is essential to consider the implications of removing pathogens from circulation given the potential for closely related animal viruses to mutate rapidly and fill the empty niche. This is particularly important since zoonotic infections are often associated with higher levels of morbidity and mortality in new hosts. Natural outbreaks in monkeys prove canine distemper virus (CDV) can jump the carnivore/primate species barrier making this an excellent system to study the molecular mechanisms involved in the adaptation process. These risks are borne out by the recent detection of novel bat paramyxoviruses, morbilliviruses and human coronaviruses illustrating the importance of understanding general mechanisms governing adaptation of animal pathogens to humans.
Morbilliviruses are highly infectious respiratory pathogens that cause profound immune suppression and have the propensity to cause large disease outbreaks in naïve populations. Measles virus (MV) is an important cause of childhood disease which in rare cases causes fatal

encephalitis. In contrast, CDV is much more neurotropic, much more likely to jump species and consequently are much more lethal. This is all the more likely given that morbilliviruses use common receptors, CD150, which explains the lymphotropism of the virus and PVRL4 which explains the epitheliotropism. Any animal morbillivirus could be highly pathogenic if it adapted to use the human receptor more efficiently. While morbilliviruses are similar enough for the MV vaccine to provide cross-protection, the public health community is currently considering global eradication of measles. If this is achieved it will likely result in significant drops in measles vaccination rates and thus greater population-level risk of zoonotic infections.

Traditional forward genetics and molecular biological-based reverse genetics approaches have been used to address these questions using viruses which express enhanced green fluorescent protein (EGFP) from an additional transcription unit inserted into the virus genome. The strength of this approach is that is removes the 'blind-passage' aspect from adaptation studies as, even in the absence of overt cytopathic effect, it is possible to determine the optimal time to passage the virus-infected cells. This is due to the continual expression of EGFP in infected cells which permits sensitive monitoring of the infection over time. Sequential passage allows the identification of mutations in key virus proteins which permit the CDV to adapt to human (h) CD150 and hPVRL4. These mutations have been located in the three-dimensional structure of the hemagglutinin (H) glycoprotein. Two interact directly with hCD150 and the others are internal in the glycoprotein. Conversely it is possible to introduce mutations into the glycoprotein which ablate natural receptor use, for example recombinant viruses which no longer utilize hPVRL4 have been recovered. These viruses help to dissect the mechanisms of virus-to-cell and cell-to-cell spread using *in vitro*, *ex vivo* and *in vivo* models.

O062

Dipeptidyl peptidase-4 is a functional receptor for the emerging human coronavirus-EMC

H. Mou[1],B.J. Bosch[1], V.S. Raj[2], S.L. Smits[2], D.H.W. Dekkers[3], M.A. Müller[4], R. Dijkman[5], D. Muth[4], J.A.A. Demmers[3], A. Zaki[6], R.A.M. Fouchier[2], P.J.M. Rottier[1], A.D.M.E. Osterhaus[2], B.L. Haagmans[2]

[1]*Utrecht University, Infectious diseases & Immunology, Division Virology, Utrecht,* [2]*Viroscience Lab, Erasmus Medical Center, Rotterdam,* [3]*Proteomics Department, Erasmus Medical Center, Rotterdam,* [4]*Institute of Virology, University of Bonn Medical Centre, Bonn, Germany,* [5]*Institute of Immunobiology, Kantonal Hospital St. Gallen, St. Gallen, Switserland,* [6]*Virology Laboratory, Dr Soliman Fakeeh Hospital, Jeddah, Saudi Arabia*

Human coronaviruses (CoVs) cause mild upper respiratory tract disease but may be associated with more severe pulmonary disease in immunocompromised individuals. SARS-CoV on the other hand caused severe lower respiratory disease with nearly 10% mortality and evidence of systemic spread. Recently, another coronavirus (HCoV-EMC) was identified in patients with severe and sometimes lethal lower respiratory tract infection.

Viral genome analysis revealed close relatedness to CoVs found in bats. Here we identify dipeptidyl peptidase-4 (DPP-4) - also known as CD26 - as a functional receptor for HCoV-EMC. DPP-4 specifically co-purified with the receptor binding S1 domain of the HCoV-EMC spike protein from lysates of susceptible Huh-7 cells. Antibodies directed against DPP-4 inhibited HCoV-EMC infection of primary human bronchial epithelial cells and Huh-7 cells. Expression of human and bat (*Pipistrellus pipistrellus)* DPP-4 in non-permissive COS-7 cells enabled infection by HCoV-EMC. The use of the evolutionary conserved DPP-4 protein from different species as a functional receptor provides clues about HCoV-EMCs host range potential. In addition, it will contribute critically to our understanding of the pathogenesis and epidemiology of this emerging human CoV, and may facilitate the development of intervention strategies.

O063

Ferreting out influenza H5N1 quasispecies evolution during human infection by whole genome deep sequencing of clinical specimens from infected patients

M.R.A. Welkers[1], H.A. Pawestri[2], R.E. Jeeninga[1], M. Crusat[1], V. Setiawaty[2], O.D. Sampurno[2], M.D. de Jong[1]

[1]*Academic Medical Centre, Dept. of Medical Microbiology, Amsterdam,* [2]*Ministry of Health National Institute of Health Research and Development, the Republic of Indonesia, Jakarta, Indonesia*

Global concerns persist that highly pathogenic avian influenza A/H5N1 viruses may evolve towards efficient transmission between humans, hence causing a severe influenza pandemic. Recent animal experimental work suggests the requirement of only a few amino acid changes for this to occur. Surveillance for genetic changes, especially during human H5N1 infections, thus remains essential for pandemic risk assessment. However, much crucial information is lost using current routine approaches since these are usually based on Sanger sequencing (which does not detect minority variants) of viruses grown in cell culture (which may select for unrepresentative variants). To circumvent these pitfalls and gain more detailed insight into H5N1 evolution during human infection, we applied whole genome next generation deep sequencing to 39 stored clinical specimens obtained from 31 H5N1-infected patients from Indonesia between 2006 and 2011, of whom 30 patients had died. From 7 patients multiple samples were available from either different time points and/or different sampling sites (e.g. upper/lower respiratory tract).

On average, a 76.1% (57.7% - 90.2%) sequence coverage of the H5N1 genome was achieved with a minimal depth of 50 nucleotides per position (mean 704, range 149 - 2310). We observed a number of minority variants at relevant amino acid positions, including resistance-conferring H275Y in neuraminidase (12% minority in a patient after 1 day of oseltamivir treatment) and virulence-associated E627K in PB2 (2-15% in 3 patients). At several HA positions previously implied by Herfst et al. and Imai et al. as important for air-borne transmission between ferrets, we observed amino acid changes in minority populations (< 10%) in several patients but to different residues than described. Our data indicates that minority variants emerge during human H5N1 infections that are potentially associated with adaptive evolution. A more detailed overview of quasispecies variation and minority variants detected will be presented.

O064

The use of whole-genome sequence data to detect recent homologous recombination events in *Enterococcus faecium*

M. de Been[1], W. van Schaik[1], L. Cheng[2], J. Corander[2], R.J. Willems[1]

[1]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*University of Helsinki, Dept. of Mathematics and Statistics, Helsinki, Finland*

Introduction: Before the 1990s the vast majority of *enterococcal* hospital-acquired infections (HAI) were caused by *Enterococcus faecalis*, but since then, *enterocccal* HAIs have been increasingly associated with *E. faecium*. Nowadays, *E. faecium* HAIs are as common as *E. faecalis* HAIs. Reasons for the increased occurrence of *E. faecium* HAIs include the rapid acquisition of genes conferring resistance to clinically important antibiotics by *E. faecium* and the emergence of specific *E. faecium* sub-populations that seem to have adopted a hospital-specific lifestyle. In this study, we focused on the impact of genomic recombination on the *E. faecium* population structure and its role in the acquisition of genes important for the adaptation to hospital-associated environments.

Methods: Whole-genome sequences of 34 *E. faecium* strains, including those of 3 newly sequenced strains from a highly successful hospital-associated sub-population, were analysed for the presence of recombinogenic regions using the previously described algorithm BratNextGen.[1] This algorithm was applied on the *E. faecium* core genome alignment. Effects of recombination on the *E. faecium* population structure were analysed by comparison of phylogenetic trees, pre- and post-filtered for recombination signals. The taxonomic sources of recombinogenic sequences were estimated by building separate phylogenetic trees for each recombination event detected and by comparing these sub-trees to an *E. faecium* reference phylogeny.

Results and Conclusion: Of the original 1.2 million positions in the *E. faecium* core genome alignment, 0.5 million (44%) were predicted to have been affected by recent recombination events in at least one strain. On the SNP level, this rate was comparable with 38,665 of the original 85,488 positions (45%) identified as being recombinogenic. The foreign genomic elements that have entered *E. faecium* appear to have had a major impact on its evolution. One important finding was that no recombinogenic signals were detected in one of the two major *E. faecium* clades (clade B) containing most of the *E. faecium* human gut commensals. However, (ancestors of) these gut commensals appeared to have formed the most important reservoir for donating foreign DNA to the second major *E. faecium* clade (clade A), which contains most of the hospital-associated isolates. Functional analysis and mapping of recombinogenic sequences to the completely sequenced (circular) genome of strain Aus 0004, revealed that a large gene cluster involved in cell envelope biosynthesis and modification (epa locus) is mainly recombining in a specific and successful lineage of hospital-associated *E. faecium* strains. Epa loci are thought to interfere with detection of pathogenic bacteria by the host immune system. This finding may thus point to an important recombination-driven, adaptive mechanism for clinical *E. faecium* strains.

References

1. Marttinen, et al. Nucleic Acids Research. 2012;40:1- e6.

O065

Utility of whole genome sequencing of *Mycobacterium tuberculosis* in the molecular epidemiology of tuberculosis

D. van Soolingen[1,2], J. Bryant[3], A.C. Schürch[1,7], H. van Deutekom[4], A. Kiers[5], S. Bentley[3], J. Parkhill[3], S.A.F.T. van Hijum[6], J.L. de Beer[1]

[1]*Tuberculosis Reference Laboratory, National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Bilthoven,* [2]*Dept. of Clinical Microbiology and Dept. of Lung Disease, Radboud University Nijmegen Medical Centre, Nijmegen,* [3]*Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom,* [4]*Dept. of tuberculosis control, Public Health Service, Amsterdam,* [5]*Dept. of Tuberculosis Control GGD Fryslân*[3]*, Leeuwarden,* [6]*Radboud University Nijmegen Medical Centre/NCMLS, Centre for Molecular*

and Biomolecular Informatics, Nijmegen, [7]Dept. of Virology, Erasmus Medical Center, Rotterdam

DNA fingerprinting of *Mycobacterium tuberculosis* was introduced in the early 1990s and has revolutionized our knowledge on the epidemiology of tuberculosis (TB). In standard typing techniques like IS6110 restriction fragment length polymorphism (RFLP) typing, 24-loci variable number of tandem repeat (VNTR) typing, and spoligo typing, only a minor part of the DNA polymorphism is visualized. Therefore, in recent years the possibilities of whole genome sequencing (WGS) were explored. To study whether WGS would yield a higher resolution in the epidemiology of tuberculosis than the currently used typing methods, three *M. tuberculosis* isolates of the Harlingen outbreak, that occurred in 1993 and spread until 2008 in the Netherlands to 110 people, were subjected to WGS. The observed genomic turnover in the Harlingen outbreak strains was very low, but the presence of in total 9 SNPs divided the large RFLP cluster in transmission chains that matched the assumptions of the Municipal Health Service. This observation raised the question on the general pace of genomic change in *M. tuberculosis* over time. We therefore analyzed 199 isolates from epidemiologically linked TB cases in Amsterdam. In accordance with the observation in a TB monkey model used by another research group, we observed an average mutation rate of ~0.3 SNPs per genome per year. However, a very high degree of variation around this mean was found, and this will reduce the utility of WGS in the molecular epidemiology. Nevertheless, on basis of WGS false clustering of cases on basis of the current RFLP/VNTR typing was refuted. Therefore, we are still convinced investigation of epidemiological links between TB patients will greatly benefit from adding this high resolution typing technique. DNA fingerprinting also contributed significantly to the disclosure of the phylogeny of the *M. tuberculosis* complex. The Beijing genotype, described by the RIVM in 1995, is one of the most important genotype families disclosed so far, as it is emerging in multiple geographic areas and associated with multi-drug resistant TB. In the traditional typing methods, Beijing genotype strains seemed genetically highly conserved and this suggested they possess selective advantages over other strains, e.g. the ability to circumvent BCG-induced immunity and withstand antituberculosis drugs. To study the phylogeny of the Beijing strains more reliably, WGS was applied to Beijing strains isolated in China, Vietnam and South Africa. The restricted number of SNPs observed in the Typical (modern) Beijing strains from a wide spread geographic area suggests these strains indeed constitute a monophyletic lineage and result from recent spread. SNP typing combined with RFLP typing revealed that about 80% of 1,522 Beijing strains investigated from multiple countries belong to this genetically highly conserved Beijing clone. Whether WGS could serve as a sole tool in the molecular epidemiology of TB, without the information on the DNA polymorphism associated with repetitive sequences, is still not clear.

O066

Next generation sequencing to elucidate the molecular epidemiology of pathogens

A. Mellmann
Institute of Hygiene, University Hospital Münster, Germany

Driven by the rapid development of next generation sequencing (NGS) technologies, in the near future shotgun genome sequencing of bacterial pathogens will be applied in clinical microbiology and infection control to unravel both the molecular epidemiology and further information such as the pathogenicity make-up and antibiotic resistance traits. This presentation will focus on two important foodborne pathogens, *Campylobacter jejuni* and enterohemorrhagic *Escherichia coli* (EHEC), and will discuss NGS data of these pathogens. For *C. jejuni*, core genome NGS data of 34 strains isolated from three outbreaks and sporadic cases of campylobacteriosis will be used to delineate a sequence similarity threshold for the differentiation of sporadic cases from outbreak isolates. Moreover, sequencing data will be applied to investigate mixed infections. For EHEC, results of an analysis of 32 EHEC O104:H4 strains that were detected during the large outbreak in May/June 2011 in Germany will be presented. These isolates included not only strains from individual patients either from different regions in Germany or within a single family but also up to four consecutive isolates derived from the same patient. During this investigation, NGS data is used to characterize evolutionary events during the outbreak on whole genome level to further elucidate the epidemiology of this highly pathogenic clone.

O067

Streptomyces: the beauty of the beast and its exploitation for the discovery of novel antimicrobials

G. van Wezel
Molecular Biotechnology, Institute of Biology, Leiden University, Leiden

Central in this talk is *Streptomyces*, a filamentous soil bacterium that produces half of all known antibiotics and a range of other natural products and enzymes. The beauty lies in the organism itself, with its wonderful development, and in the often colourful natural products it produces. The treasures that lie hidden in the actinomycete genomes may well be our final resource in the fight against the rapidly emerging multi-drug resistant pathogens. Development and antibotic production are highly coordinated in this complex micro-organism, and we aim to understand the global regulatory networks that determine the switch from normal growth to the developmental programme. A major control system revolves around the nutrient sensory protein DasR, which links primary metabolism to the control of antibiotic production. Key aspects of the DasR regulatory network, which is one of the largest regulons found in bacteria, will be discussed. I will also show how we apply this knowledge to develop novel approaches to wake up silent antibiotics. Our NGS-based proteomining technology thereby fascilitates rapid linkage of novel treasures to the gene clusters responsible for their production.

O068

Synthetic biology applied to the discovery and improvement of lantibiotics

M. Montalbán-López, A. van Heel, D. Mu, D. Hendriks, O.P. Kuipers
Molecular Genetics Group, University of Groningen, Groningen

Lantibiotics are a group of potent antimicrobials with potential application in therapeutics. The main characteristic of this group of ribosomally synthesized peptides is the presence in their structure of unusual amino acids such as dehydrated residues and (methyl)lanthionine among many others. Nisin is the prototype lantibiotic. During nisin biosynthesis NisB dehydrates serines and threonines in the propeptide and NisC catalyses the addition of the thiol group in cysteines to an N-terminally located dehydroamino acid to create the (methyl)lanthionine ring. NisT transports the modified prenisin across the membrane of the producer organism. The presence of the leader peptide is directing the prepeptide through all the modification machinery. The combination of these three enzymes, NisBTC, has been used for the cyclization and stabilization of biologically active peptides fused to the leader peptide of nisin (e.g. angiotensin or enkephalin).
In our lab we are exploring the modularity of lantibiotics from two different points of view. On one hand we are further developing the NisBTC system with additional enzymes from lantibiotic biosynthesis. Our purpose is the addition of new modifications that can be orthogonally implemented in a peptide 'à la carte'. Using this approach we have been able to incorporate the conversion of serine to D-alanine in nisin, a reaction catalized by the hydrogenase LtnJ from the gene cluster of lacticin 3147. We have also plugged in the system the oxidative decarboxylation catalyzed by GdmD, from the gene cluster of gallidermin, in a nisin-gallidermin hybrid.
On the other hand, we are creating a library containing more than 10,000 members of potentially active peptides by the combination of rings from existing lantibiotics, thus using the lanthionine rings as modules. The screening for bacterial inhibition of such a big library relies on the development of a high-throughput screening system in which the producer organism and the sensitive bacteria grow together in a nanoreactor. After the production of the candidate peptides, the nanoreactors in which the sensitive bacteria are killed can be sorted out from the ones where the candidate is not active. Additionally we have produced a soluble variant of the protease NisP that can be added to the screening system to remove the leader peptide therefore activating the novel lantibiotics.

O069

Chicken cathelicidins display antimicrobial activity against multiresistant bacteria without inducing strong resistance

E.J.A. Veldhuizen[1], C. Brouwer[2], A.F. Schneider[1], A.C. Fluit[2]
[1]Utrecht university, Dept. of Infectious Diseases & Immunology, Utrecht, [2]University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: The increased prevalence of multidrug-resistant (MDR) bacteria and the relatively limited development of new antibiotics pose a serious threat to public health. In chicken, especially extended spectrum bèta lactamase (ESBL) carrying *Enterobacteriaceae* are often asymptomatically present and can infect human. Due to their broad range antimicrobial activity cathelicidins and other host defence peptides, are considered as an attractive alternative for conventional antibiotics. In this study, the antimicrobial activity of three chicken cathelicidins against a broad array of multidrug-resistant bacteria was determined.
Methods: Antimicrobial activity of cathelicidins was tested against 39 bacterial strains using broth dilution assays, spot tests and colony count assays.
Induction of resistance towards cathelicidins was determined by cultivating *S. aureus* and *K. pneumoniae* for 10 days in the presence of increasing concentrations of peptide
Results: In this study, only minor differences between the three cathelicidins were observed. All three peptides showed high antibacterial activity independent of the presence of multidrug-resistance characteristics. Induction experiments using *S. aureus* and *K. pneumoniae* showed that although an increase in resistance was initially observed, susceptibility towards chicken cathelicidins remained high and no major resistance was developed.
Conclusion: The broad antibacterial spectrum of chicken cathelicidins and the lack of development of bacterial resistance towards these peptides underline the potential of chicken cathelicidins as a new alternative to antibiotic

O070

Real-time *in vivo* imaging of invasive- and biomaterial-associated bacterial infections using fluorescently labeled vancomycin

M. van Oosten[1], T. Schäfer[2], J.A.C. Gazendam[1], K. Ohlsen[2], E. Tsompanidou[1], M.C. de Goffau[1], H.J.M. Harmsen[1], L.M.A. Crane[1], K.P. Francis[3], L. Cheung[4], M. Olive[4], V. Ntziachristos[5], J.M. van Dijl[1], G.M. van Dam[1]

[1]UMCG, Dept. of Medical Microbiology, Groningen, [2]Universität Würzburg, Institut für Molekulare Infektionsbiolog, Würzburg, Germany, [3]Caliper Life Sciences, Alameda, USA, [4]LI-COR Biosciences, Lincoln, USA,[5]Technische Universität München & Helmholtz Zentrum, München, Germany

Introduction: Invasive and biomaterial-associated infections (BAI) in humans are often difficult to diagnose and treat. Guided by recent advances in clinically relevant optical imaging technologies, we explored the use of fluorescently labeled vancomycin (vanco-800CW) to specifically target and detect infections caused by gram-positive bacteria.

Methods: Vanco-800CW was obtained by conjugating vancomycin to the near-infrared fluorophore IRDye 800CW. The specificity and sensitivity of vanco-800CW binding to different bacteria was tested *in vitro*. Next, the application potential of vanco-800CW for real-time *in vivo* imaging of invasive and biomaterial-associated bacterial infections was assessed in a myositis mouse model and a human post-mortem implant model.

Results: We show that vanco-800CW can: (1) specifically detect gram-positive (in particular, *Staphylococcus aureus*) infections in a bacterial myositis mouse model, (2) discriminate bacterial infections from sterile inflammation *in vivo*, and (3) detect BAI on osteosynthetic devices in the lower leg of a human cadaver.

Conclusion: Vanco-800CW enhances diagnosis of infection with gram-positive bacteria (e.g. bacterial myositis and BAI), and is a promising candidate for early phase clinical trials. By using non-invasive fluorescence imaging in conjunction with a bacteria-specific optical probe, we hope to optimize current treatment strategies and ultimately reduce the morbidity, mortality and overall healthcare costs incurred by invasive infections and BAI.

O071

The fungi strikes back: multidrug-resistance in *Aspergillus fumigates* and agricultural use of fungicides

W.J.G. Melchers

Radboud University Nijmegen Medical Center, Dept. of Medical Microbiology, Nijmegen

The mould *Aspergillus fumigatus* can cause a spectrum of diseases in humans, ranging from allergic conditions to life-threatening invasive diseases in immunocompromised patients. The triazoles, itraconazole, voriconazole and posaconazole are the main drugs used for the management of *Aspergillus* diseases. Although until recently *A. fumigatus* was considered uniformly susceptible to medical triazoles, there are increasing reports of acquired resistance, most frequently due to single nucleotide polymorphisms in the Cyp51A-gene. In the Netherlands azole resistance was first reported in 1998 and since then the frequency has increased. Recently it was shown that azole resistance in *A. fumigatus* is endemic in the Netherlands and that patients with azole-resistant invasive aspergillosis have a probability of 88% of dying within 12 weeks of diagnosis. A highly dominant resistance mechanism was found (TR_{34}/L98H) which was present in over 90% of clinical resistant isolates. Resistance was observed in patients without previous azole exposure and TR_{34}/L98H was found in environmental *A. fumigatus* isolates. It was hypothesized that resistance may have emerged through exposure to 14a-demethylase inhibitors (DMIs). Thirty-one DMIs, that have been authorized for use in the Netherlands between 1970 and 2005, were investigated for the presence of cross-resistance to medical triazoles. Furthermore, CYP51-protein homology modeling and molecule alignment studies were performed to identify similarity in molecule structure and docking modes. Five triazole DMIs, propiconazole, bromuconazole, tebuconazole, epoxiconazole and difenoconazole, showed very similar molecule structures to the medical triazoles and adopted similar poses while docking the protein. These DMIs also showed the greatest cross-resistance and, importantly, were authorized for use between 1990 and 1996, directly preceding the recovery of the first clinical TR_{34}/L98H isolate in 1998. Through microsatellite genotyping of TR_{34}/L98H isolates we were able to calculate that the first isolate would have arisen in 1997, confirming the results of the abovementioned experiments. Azole-resistant *A. fumigatus* isolates appear to remain virulent and are capable of causing invasive disease in patients at risk. The efficacy of azole compounds against azole-resistant isolates, with different resistance mechanisms, has been investigated in experimental models of invasive aspergillosis. These indicate that the minimal inhibitory concentration (MIC) has major impact on the efficacy of the azole. This indicates that the rapid diagnosis and identification of mutations related to resistance may influence the individual patient management and the success rate of treatment. This seems even the more important as recently a second resistance mechanism TR46/Y121F/T289A has emerged in the Netherlands, following a similar pattern to that of TR34/L98H.

O072

Chest imaging and detection and diagnosis of invasive fungal disease in the immuno-compromised host

P.A. de Jong

University Medical Center Utrecht, Dept. of Radiology, Utrecht

Fungal infection is an important cause of human disease. *Aspergillus fumigatus* is estimated to cause worldwide annually > 200,000 life-threatening infections.[1]

The EORTC/MSG consensus group has proposed definitions for invasive fungal disease to advance research, but the definitions are also useful in daily practice.[2] Chest computed tomography (CT) can play an important role to proof the infection by visualising the most appropriate site for cytology or histology and it can guide the needle into the suspected lesion. Chest CT also plays an important role for establishing a probable/possible diagnosis. CT criteria are dense well-circumscribed lung lesion(s) with or without a halo and lesions with an air-crescent and/or cavity. Chest radiographs are often normal (in the early stages) of invasive fungal lung disease and chest CT is clearly more sensitive.[3] Some chest CT findings are non-specific, even in the setting of an immuno-compromised host,[4] which might be related to the timing of the CT scan.[5]

CT imaging is commonly done at day five of persistent fever of unknown origin despite antibiotic therapy and during follow-up of proven or probable invasive fungal infections. Lesions can grow in size even under effective anti-fungal therapy and cavitation is thought to be related to bone marrow recovery and effective therapy.

Most research has been done with older type of CT technology and recent advances in acquisition speed, image quality and dose reduction may allow a new role for CT in the diagnostic strategy of possible/probable invasive fungal disease. There is still a clear need for high-quality diagnostic studies related to imaging and invasive fungal disease.

References

- Brown GD, et al. Hidden Killers: Human Fungal Infections. Science Translational Medicine. 2012;4:1-9.
- Pauw B de, et al. Revised Definitions of Invasive Fungal Disease from the EORTC/MSG Consensus Group. Clin Infect Dis. 2008;46:1813-21.
- Heussel CP, et al. Pneumonia in Febrile Neutropenic Patients and in BMT Recipients; Use of HRCT. J Clin Oncol. 1999;17:796-805.
- Escuissato DL, et al. Pulmonary infections after bone marrow transplantation: high-resolution CT findings in 111 patients. AJR. 2005;185:608-615.
- Caillot D, et al. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. J Clin Oncol. 2001;19:253-9.

O073

Diagnosis of *Pneumocystis jirovecii* pneumonia (PJP) and differentiation between active PJP and colonization in immunocompromised patients with real-time PCR

R. te Witt, C. Croes, J.J.A. van Kampen, A.G. Vonk, J.J. van Hellemond

Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam

Introduction: The golden standard for the diagnosis of *Pneumocystis jirovecii* pneumonia (PJP) is microscopic demonstration of *P. jirovecii* in Bronchoalveolar lavage (BAL) samples. However, this laborious method lacks sensitivity and requires well-trained technicians. In this study, we assessed the value of semi-quantitative real-time PCR (Alanio et al, CMI, 2010) for the diagnosis of PJP.

Materials and methods: Two hundred and fifty-one BAL fluids from 232 immunocompromised patients presenting with signs of pneumonia were tested retrospectively.

Results: All 68 microscopic PJP positive samples tested positive by PCR (sensitivity 100%). Among 183 microscopic PJP negative samples, 134 samples tested negative by PCR, supporting exclusion of PJP (negative predictive value 100%). 49 (25.1%) samples were positive by PCR, probably suggesting *P. jirovecii* colonization. Using an upper cut-off Ct-value of 32 and a lower cut-off of 29, we were able to discriminate active pneumonia from colonization for 95% of the patients, with a small intermediate zone between these two cut-off values.

Conclusion: We conclude that this real-time PCR based strategy provides sensitive and specific results for the diagnosis of PJP and is a valuable tool for distinguishing active *P. jirovecii* pneumonia from colonization.

O074

The classical complement pathway induces phagocytosis of *Aspergillus fumigatus*

S.G.E. Braem[1], J.J.P.A. Cock[2], H.A.B. Wösten[2], J.A.G. van Strijp[1], P.J.A. Haas[1]

[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]Utrecht University, Microbiology and Kluyver Centre, Utrecht

Aspergillus fumigatus is a fungal pathogen causing different types of lung infections like allergic bronchopulmonary aspergillosis, aspergilloma and invasive aspergillosis. Especially immunocompromised patients like haematological and transplant patients are affected and infection in these patients often leads to death. Although the immunocompromised state of the patient is an important factor leading to disease, infection dynamics and pathogenesis of *A. fumigatus* are poorly understood.

The innate immune system is the first line of defence against invading micro-organisms and is essential in clearing fungal infections. Upon invasion different opsonins recognise the foreign particle and activate the complement system via three different routes. C1q binds to immunoglobulines bound to the micro-organism and initiates the classical pathway leading to cleavage of C4 into C4b. The lectin pathway is initiated when mannose-binding lectin (MBL) binds to sugar structures present on the surface of micro-organisms and results also in the cleavage of C4. C4b is deposited on the outer surface and forms a

C3 convertase C4bC2a resulting in cleavage of C3 in C3a and C3b. The alternative pathway acts as an amplification loop and can be activated via spontaneous cleavage of C3 in C3b, which is deposited on the outer surface of the micro-organism. C3a is a chemoattractant resulting in recruitment of phagocytes to the site of infection. Phagocytes recognise the opsonised micro-organism via complement receptors, binding C3b, and Fc receptors, recognising the Fc tail of bound immunoglobulines, resulting in activation of the phagocyte, uptake of the micro-organism and subsequent killing within the phagolysosome.

The human body encounters different morphotypes of *Aspergillus fumigatus*. Dormant conidia are inhaled and enter the lung alveoli. When infection is not cleared properly, conidia swell, germinate, and form hyphal structures invading human tissue. Current knowledge demonstrates that all morphotypes activate the alternative pathway, whereas complement activation on hyphae is partly classical pathway dependent. Dormant conidia of some strains activate the classical pathway in absence of MBL. However, when MBL is reconstituted these strains activate only the alternative pathway. Phagocytosis of dormant conidia is thought to be complement dependent and fully initiated via the alternative pathway.

Here we show that C3b and IgG is deposited on all morphotypes and that phagocytosis by neutrophils is complement dependent. Heat-inactivated sera, without any complement activity but intact immunoglobulines, or solely IgG does not induce any phagocytosis. Different complement depleted sera demonstrate that swollen conidia activate complement only via the classical pathway, suggesting that immunoglobulines are essential for initiation of the complement system, which consequently is crucial for phagocytosis. Indeed, we show that C1q neutralising antibodies prevent phagocytosis of swollen conidia completely.

In conclusion, the present data are in strong contrast with current literature, where alternative pathway is the initiator of complement activation and subsequent phagocytosis. Here we demonstrate that the initiation of complement on swollen conidia is dependent on immunoglobulines deposited on conidia, leading to attachment of C1q, and initiation of the classical complement pathway. The corresponding C3 cleavage results in C3b deposition on the outer surface inducing phagocytosis.

O075

Global progress in TB vaccine development

H. McShane

The Jenner Institute, University of Oxford, United Kingdom

Tuberculosis (TB) remains a significant cause of mortality and morbidity throughout the world and better control measures are urgently needed. Vaccination is the most cost-effective way to control any infectious disease epidemic. The only licensed vaccine against TB, BCG, confers good protection against disseminated disease but fails to protect against pulmonary disease in the developing world.

Over the last ten years, much progress has been made in TB vaccine development, and there are now 16 candidate TB vaccines being evaluated in clinical trials. The two main strategies are either to replace BCG with an improved whole organism mycobacterial priming vaccine, or to develop a subunit booster vaccine, to be given some time after BCG. MVA85A is a recombinant Modified Vaccinia virus Ankara expressing the immunodominant mycobacterial antigen 85A. This vaccine was developed in Oxford, can improve BCG induced protection in mice, guinea pigs, non-human primates and cattle, and is the most clinically advanced new TB vaccine in development. In a series of phase I and IIa clinical trials in the UK, The Gambia, Senegal and South Africa, MVA85A has been demonstrated to be safe and highly immunogenic. Two large phase IIb efficacy trials are currently underway to evaluate the protective efficacy of MVA85A, one in BCG-vaccinated South African infants and one in HIV-infected adults in South Africa and Senegal.

Despite this progress in the field, there remain significant hurdles to the development of new TB vaccines. The lack of an immunological correlate of protection with which to select which vaccines should enter into large, expensive efficacy trials is a major challenge. In other fields of vaccine development, human challenge models can facilitate vaccine development. Whilst we cannot challenge humans with virulent *Mycobacterium tuberculosis*, we are working on a BCG human challenge model to evaluate *in vivo* mycobacterial suppression. We are also developing an *in vitro* mycobacterial growth inhibition assay to further facilitate vaccine development. The mucosal route of vaccine delivery has shown promise in mice. We are currently evaluating the safety and immunogenicity of mucosal administration of MVA85A in humans in a phase I clinical trial.

O076

From *Mycobacterium tuberculosis* antigen discovery to new TB subunit vaccines in humans

T.H.M. Ottenhoff

Leiden University Medical Center, Dept. of Infectious Diseases, Leiden

Mycobacterium tuberculosis (Mtb) is responsible for almost 2 million deaths and 9 million new cases every year. BCG, the only vaccine available against tuberculosis (TB), induces highly variable protection against TB, and better TB vaccines are urgently needed. Improved TB vaccines need to induce long-term immunity against Mtb, particularly Th1 immunity and IFN-γ secretion which are critical components of the protective response.

We have recently evaluated the safety and immunogenicity of a new TB subunit candidate vaccine. This vaccine consisted of a recombinant fusion protein Ag85B-ESAT6 mixed with IC31, a new Th1-promoting adjuvant (van Dissel et al, Vaccine 2010, 2011). Ag85B and ESAT6 are two highly immunogenic early phase secreted proteins of Mtb, shown to be protective in various animal models of acute TB infection. The vaccine did not cause local or systemic adverse effects, besides some local transient soreness directly after injection, but elicited strong antigen specific T-cell responses against both the Ag85B and ESAT6 as measured by IFN-γ ELISpot. Remarkably, strong responses persisted through 2 years follow-up, indicating a substantial memory response in the vaccine recipients.

It has recently become clear that Mtb displays significant phase-related variation in gene expression during the different stages of infection. Following the early phase of infection in which the expression of antigens like ESAT6 and Ag85B is high, Mtb then down regulates the expression of many of its secreted antigens, and induces the expression of a set of latency genes. These latter genes allow Mtb to establish a phase of chronic, persistent infection. We have hypothesized that antigens encoded by these genes may provide novel targets for vaccination, and that they may direct the immune system to a different reservoir of Mtb bacteria in the human body. In a series of studies we showed that latency antigens encoded by the hypoxia inducible Mtb DosR regulon are highly immunogenic for human Th1 T-cells in different human populations (Leyten et al, Micr Inf 2006; Lin et al, Inf Immun 2007; Schuck et al, PLoS ONE 2010, Black et al, Clin Vacc Immun 2009, Ottenhoff et al, PLoS Pathogens 2012). Thus, these antigens provide interesting new candidates for multistage subunit TB vaccines, as will be discussed.

O078

Post-exposure subunit vaccination against chronic mycobacterial infection in a natural host

A.P. Koets, W. Santema, V. Rutten, R. Segers, J. Poot, S. Hensen, J. Heesterbeek

Utrecht University, Faculty of Veterinary Medicine, Utrecht

The control of chronic bacterial diseases with high prevalence in endemic areas would strongly benefit from availability of post-exposure vaccines. The development of these vaccines against mycobacterial infections, such as tuberculosis, is hampered by lack of experience in natural hosts. Paratuberculosis in cattle is both a mycobacterial disease of worldwide importance and a natural host model for mycobacterial infections in general. The present study showed beneficial effects of therapeutic heat shock protein 70 (Hsp70) vaccination in cattle with naturally acquired chronic infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Vaccination-induced protection was associated with antibody responses, rather than with induction of specific T-helper 1 cells. Targeted therapeutic post-exposure vaccination complementary to selective use of antibiotics could be an effective approach for control of chronic mycobacterial infections. Based on high Hsp70 amino acid sequence conservation between mycobacterial species, and immunopathogenic parallels, this post-exposure immunization approach may aid control of both human and bovine tuberculosis.

O079

STEC-ID-net: a feasibility study for a new STEC diagnostic strategy

A.M.D. Kooistra-Smid[1], R.F. de Boer[1], J.W.A. Rossen[2], A. Ott[1], A.W. Friedrich[2]

[1]*Laboratory for Infectious Diseases, Dept. of Medical Microbiology, Groningen,* [2]*University of Groningen, University Medical Center Groningen, Dept. of Medical Microbiology, Groningen*

Shiga toxin-producing *E. coli* (STEC) can cause a broad spectrum of diseases comprising mild gastro-enteritis, hemorrhagic colitis and the hemolytic uremic syndrome (HUS). In particular, STEC strains belonging to serogroups O26, O103, O104, O111, O121, O145 and O157 are known causative agents of outbreaks and serious illness and are usually called HUS-associated *E. coli* (HUSEC).

Since 2008 the LCI-guidelines for detection of STEC in the Netherlands have changed, based on results of a nationwide study.[1] Detection of STEC in the Netherlands was traditionally limited to the detection of serogroup O157, but laboratories were encouraged to use techniques (such as PCR) targeting Shiga toxin-coding genes, enabling detection of non-O157 serogroups.

Currently, approximately 1/3 of public health laboratories in the Netherlands use PCR diagnostics for detection of STEC (stx1/stx2), but 2/3 still use only (CT-) SMAC culturing for the detection of STEC O157. Using PCR diagnostics for STEC, the detection rate of STEC increased significantly[2] leading to a major increase in work burden at the Public Health Services (PHS). On the other hand, laboratories that only use (CT-) SMAC for detection of STEC still are not able to detect public health relevant non-O157 infections. Anyway, the notification of STEC in theNetherlands rose 5-fold from 2008 to 2011. This is due to the increased use of PCR-based diagnostics but probably also to a real increase of infections.

The variability in diagnostic assays, the unclear infection control and public health impact as well as the variability of public health action demand improvement of STEC diagnostics and surveillance. The PHS requires additional information on the isolate and a molecular risk assessment.
At the 2012 Scientific spring meeting of the KNVM& NVMM we proposed a three-level diagnostic approach for STEC-infection in the Netherlands using an established diagnostic scheme.[3] Based on this, we implemented and validated a partly modified algorithm enabling a fast discrimination of hypervirulent HUSEC from less virulent STEC. Furthermore, it enables the detection of viable STEC in stool samples. The first single center pilot-analysis shows an improved detection of STEC and HUSEC. In order to prove feasibility, the STEC-ID-net was set up. A prospective cohort study will be performed in 2013 to achieve data on which recommendations can be based for new STEC diagnostics in the Netherlands. Within STEC-ID-net a number of institutes (LvI, STAR-mdc, UMCG, PHS Groningen, PHS Drenthe, PHS Rotterdam-Rijnmond and RIVM) collaborate and combine their expertise in order to optimize the identification of pathogenic STEC and HUSEC, STEC surveillance and the public health response.

References

1. Duynhoven van, et al. CMI. 2008;14:437-45.
2. Boer de, et al. JCM. 2010;48:4140-6.
3. Friedrich, et al. JCM. 2004;42:4697-701.

O080

CCGE Study gastro-enteritis – first results

L.E.S. Bruijnesteijn van Coppenraet[1], G.J.H.M. Ruijs[1], W.A. van der Reijden[2], T.G. Mank[2], L. Mulder[3], A.G.M. van der Zanden[3], T.A. Schuurs[4], J. Weel[4]
[1]Isala Klinieken, LMMI, Zwolle, [2]Streeklaboratorium, Haarlem, [3]Labmicta, Enschede, [4]Izore, Leeuwarden

With the application of highly sensitive molecular diagnostics of infectious gastro-enteritis (GE), more and more questions are rising concerning the clinical value of a positive detection. Either because of the high positivity rate of detections with low pathogen load but also because of the detection of organisms for which limited clinical data are present.
During the last two years, in four different regions of the Netherlands stool samples were collected from patients who visited their general practitioner because of gastro-intestinal complaints. For each stool sample of a patient, a matched control sample was asked from a patient, who visited the general practioner for other reasons than gastro-enteritis. Matching criteria were age group, gender, season, and region.
The main objectives of the study are:
1) to determine the difference in detection rate of putatitive gastrointestinal bacterial and parasitic pathogens between cases and controls; and
2) analysing the clinical value of semi-quantitative results for positive detections.
A total of 2700 materials was collected by the four participating laboratories, comprised of 1550 GE case stool samples and 1150 control samples.
All samples were tested for the presence of *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium parvum/hominis*, *Entamoeba histolytica*, *Campylobacter* spp, *Salmonella* spp, Pathogenic *Yersinia enterocolytica*, pathogenic *E. coli*, *Shigella* spp and toxinogenic *Clostridium difficile* by multiplexed real-time PCR.
Materials are being processed momentarily. However, preliminary results show a particular high prevalence of *D. fragilis*, enteropathogenic *E. coli* (EPEC) and Shiga-like toxigenic *E. coli* (STEC) in healthy controls compared with GE cases.
More results will be presented at the session 'Finding the needle in the haystack: molecular diagnostics of gastro-enteritis', during the NVMM springmeeting 2013.

O081

Molecular diagnostics of intestinal parasites

T.A. Schuurs[1], A.J. Stellingwerff[1], S. Mulder[1], J.J. van Hellemond[2], J. Weel[1]
[1]Izore, centre for infectious diseases Friesland, Dept. of Molecular Microbiology, Leeuwarden, [2]Erasmus University Medical Center & Rotterdam Harbour Hospital, Dept. of Medical Micobiology and Infectious Diseases, Rotterdam

Implementation of molecular screening for pathogenic parasites causing gastro-enteritic complaints, has replaced microscopical TFT analysis in many medical microbiology (MM) laboratories in the Netherlands. The advantages of molecular screening for these intestinal parasites, compared to classical microscopy, are well known: quick result, highly sensitive and specific, and less laborious.
Currently, laboratories are confronted with several 'next-step' issues determining the quality of molecular diagnostics of gastro-enteritis. First, the number of samples from patients with symptoms of gastro-enteritis is substantial and still increasing. Together with the extensive number of different pathogens to be analyzed in each sample, automated sample processing has become essential. In our laboratory the implementation of the FLOW-concept (Roche), in combination with a 384-wells PCR set-up, has led to increased capacity, reliable sample-tracking and decrease in hands-on time. Also, a less laborious pre-treatment step, having no negative effect on analytical and clinical sensitivity, was developed enabling automated sample-handling.
Although molecular screening for gastro-enteritic parasites is performed by an increasing number of MM laboratories, quality control panels to test the laboratories individual performances were not available yet. Starting as a pilot, a molecular quality control panel organized by the parasitology section of the SKML is becoming official in the course of 2013. Characteristics of this panel will include: clinical feces samples, challenging loads and clinically relevant targets. Also, QCMD is planning a quality control panel for 2013.
Introduction of molecular screening, in searching infectious causes of gastro-enteritis, has in some cases led to enormously increased sensitivity. Therefore, interpretation of PCR-generated results may be quite challenging. For instance, what is the clinical relevance of *Dientamoeba fragilis* as we find PCR-positivity in 40% of the samples. Therefore, initiatives such as the multi-centre case-control gastro-enteritis (ccge) study will help to better understand the results generated with molecular techniques, detecting DNA of pathogenic parasites in fecal samples of patients suffering from gastro-enteritis.

O082

Molecular diagnostics of intestinal parasites; implications of the introduction in routine clinical practice

T.G. Mank
Regional Laboratory of Medical Microbiology & Public Health Haarlem, Parasitology, Haarlem

In parasitology, examination of stool specimens is the most frequently performed laboratory procedure. In the Netherlands, standard methods include microscopic examinations of (permanently) stained or wet preparations made from either fresh or SAF preserved stool specimens (with and without concentration). Because intestinal parasites are shed intermittently, patients are asked to deliver multiple stool specimens collected on consecutive days for examination (usually by using the so-called TFT). With the use of the described methods in combination with specifically trained and well experienced microscopists a broad spectrum of helminth and protozoal species can be detected in stool specimens.
There is an increasing trend in theNetherlands towards the routine use of non-morphological methods (immunoassay based and PCR techniques) for diagnosis of intestinal parasitic infections; either combined with or as a replacement of microscopic techniques.
A major benefit of PCR-based methods is the exquisite sensitivity; in particular when compared to the sensitivity of microscopy performed on a single stool specimen. Furthermore, PCR-based methods are less time consuming as compared to microscopic techniques, and with the use of PCR morphologically identical species or genotypes can be distinguished. On the other hand, PCR-based methods require specific laboratory conditions and equipment and are, at the moment, rather expensive for patients. Finally PCR-based methods are species specific whereas with microscopy, a broad range of expected (and unexpected) parasitic life forms can be detected simultaneously. Therefore a parasitological stool examination should preferably comprise methods 'from both worlds'.
Multiplex PCR tests for panels of gastrointestinal pathogens (*e.g. G. lamblia*, *Cryptosporidium* sp, *E. histolytica* and *D. fragilis*) can be used as a screening tool. However in certain patient groups (e.g. in case of travelling abroad, and immunocompromised patients) the current PCR-panels frequently used in routine clinical practice are too limited and microscopic techniques should be performed as well. Furthermore, microscopic confirmation of positive PCR results remains necessary until the clinical relevance of unconfirmed positive PCR-results is clear.
While molecular techniques may most certainly aid diagnosis, any loss of microscopy skills would be to the detriment of parasitology services in the Netherlands

O083

Direct molecular analysis of polymicrobial infections by endogenous microbiota with IS-pro

M. Hoogewerf, C.W. Ang, P.H.M. Savelkoul, A.E. Budding
VU Medical Center, Dept. of Medical Microbiology and Infection Control, Amsterdam

Introduction: Abscesses, especially liver and brain abscesses, are often caused by multiple bacterial pathogens derived from the endogenous (commensal) microbiota. Traditional culturing techniques may not be able to detect all bacterial species present in these complex infections, as many commensal species have now been shown to be highly refractory to cultivation. Of relevance for clinical application, cultivation techniques may also yield negative results after antibiotics have been administered to a patient.
Here we investigate the application of a new molecular technique, IS-pro, in the analysis of (poly)microbial infections from pus specimens derived from abscesses and punctures from different locations. The results of IS-pro were compared to traditional culturing techniques.
Methods: IS-pro is a new PCR-based profiling technique that combines bacterial species differentiation by the length of the 16S-23S rDNA interspace (IS) region with instant taxonomic classification by phylum-specific fluorescent labelling of PCR primers. The IS region is

extremely variable in size and sequence, even within closely related taxonomic groups, making it very suitable for analysis of complex communities.
We prospectively collected 137 human clinical specimens of abscesses (100) and punctures (37) from different locations and analysed them with IS-pro and bacterial cultivation techniques.
Results: In 43/137 (31%) clinical specimens, no bacteria were cultured. IS-pro detected bacterial species in 35% of culture negative specimens.
51 of the 94 (54%) culture positive samples showed identical results in culture and IS-pro. In 30 samples (32%) IS-pro detected the same bacterial species as were found with culture, but also a diverse array of additional bacterial species that were not found by cultivation. Often > 1 additional species was detected per sample.
In 5/94 culture positive samples no bacterial species, or less species than cultured were detected. In 8/94 culture positive samples other bacterial species were detected than cultured. With Is-pro we also detected a *Mycobacterium tuberculosis* in 2 pus specimens obtained from a patient with a spondylodiscitis, culture and PCR showed the same results.
Conclusion: The IS-pro technique showed to be more sensitive than cultivation in the analysis of polymicrobial infections. In 33% of the clinical specimens more species were detected with IS-pro than with cultivation. This is not only relevant as a clinical tool with improved sensitivity, but also very much in a more fundamental sense to come to a better understanding of polymicrobial infections by endogenous microbiota.
Finally, IS-pro is applicable as a high-throughput tool with a short turnover (1 day) time which makes it a highly interesting alternative to cultivation in the analysis of (poly) microbial infections.

O086

Exposure of *Aspergillus fumigatus* to methylprednisolone results in increased expression of cell wall genes associated with virulence

D. Bathoorn[1], S. Braem[1], P. Krijgsheld[2], G.J. van Veluw[2], J.A.G. van Strijp[1], H.A.B. Wösten[2], P.J.A. Haas[1]
[1]*UMCU, Dept. of Medical Microbiology, Utrecht,* [2]*University of Utrecht, Microbiology and Kluyver Centre, Utrecht*

Introduction: Treatment with corticosteroids is associated with increased risk on invasive aspergillosis. There are indications that the effects on the immune system are not the only explanation for the increase risk on invasive aspergillosis with corticosteroid use. We have found that exposure to methylprednisolone (MP) causes a phenotypic change in *Aspergillus fumigatus 293* (AF293) growth. Effects of MP on AF293 whole genome mRNA expression were assessed to identify molecular pathways by which it acts. Cell wall associated genes encoding for hydrophobin, melanin, galactomannan, and ergosterol were of specific interest to explain the phenotypic change.
Methods: Liquid submerged cultures were incubated for 8 hours with $8x10^6$ AF293 spores ml-1 in complete medium at 37 °C, with and without the addition of 1 M MP. RNA of AF293 was isolated and used for RNA sequencing. T-tests were performed on mean reads per kb per million reads of triplicate experiments in both groups ($p < 0.05$, FDR < 0.01). Genes were regarded as differentially expressed genes (DEGs) when the average 2log mean value was > 1, or < -1. A hypergeometric test (with Bonferroni corrected p-value < 0.05) was used to find significantly enriched gene otology (GO) terms in DEGs compared to the genome background.
Results: In total, 9,030 different transcripts of AF293 genes were expressed of the 9,887 described; 400 were upregulated, and 502 downregulated DEGs in MP treated AF293. GO analysis showed overrepresentation of upregulated genes annotated to oxidoreductase reactivity, iron ion binding, cell wall structure, and organic substance transport. Four of 4 genes encoding for hydrophobins were differentially upregulated in MP treated AF293, including rodA and rodB. In addition, 3 of 6 genes involved in melanin synthesis were differentially expressed. Of these, 2 were upregulated DEGs (arp1 and arp2), and 1 was a downregulated DEG (ayg1). We detected 3 genes involved in galactomannan expression: galactomannoprotein man pol1, and antigenic cell wall galactomannoprotein were upregulated DEGs; OCH1 was indifferently expressed. None of 13 annotated genes to ergosterol synthesis were DEGs.
Conclusion: Exposure of AF293 to MP causes changes in cell wall structure by upregulation of hydrophobin, melanin, and galactomannan genes. These genes are associated with virulence, and defence against oxidative stress and toxic compounds. Future experiments using functional assays will give insight in the clinical relevance of these results.

O087

Caspofungin does not skew the early cytokine balance in experimental invasive pulmonary Aspergillosis

J.M. Refos, P.E.B. Verwer, M.T. ten Kate, A.G. Vonk, H.A. Verbrugh, I.A.J.M. Bakker-Woudenberg, W.W.J. van de Sande
Erasmus MC, Dept. of Medical microbiology, Rotterdam

Introduction: *Aspergillus fumigatus* causes life-threatening invasive pulmonary aspergillosis (IPA) in immunocompromised patients. -Glucan is an essential cell wall component and exhibits potent pro-inflammatory properties as a fungal pathogen-associated molecular pattern (PAMP). Caspofungin (CAS), an echinocandin, inhibits the biosynthesis of 1,3 -glucan and is used as salvage therapy of IPA patients.
Recently, *in vivo* and *in vitro* data suggested an echinocandin paradoxical effect showing a decrease in antifungal effectiveness at a relatively increased CAS dosage. Also, whereas high CAS concentrations can lead to a -glucan synthesis stop, low CAS concentrations increase -glucan exposure, skewing the immune response to either an anti- or proinflammatory profile. We investigated the *in vivo* cytokine profile in neutropenic rats with IPA in response to treatment with a human pharmacokinetic-equivalent CAS dose in order to advance our knowledge of antifungal responses to CAS and potentially improve treatment strategies.
Materials and methods: In 12 neutropenic female albino RP rats, a left-sided pulmonary *A. fumigatus* infection was established. CAS was administered intraperitoneally once daily (4 mg/kg/day) starting 16 h after infection. Control animals were treated with vehicle. Blood was drawn from the tail vein on days 1, 3 and 6 after start of infection. Cytokine levels were determined in lung homogenates and serum using a bead-based flow cytometry multiplex technique against the following cytokines and chemokines: IL-1a, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17a, IL-18, IL-21, IL-22, IFN-γ, TNF-α, G-CSF, GM-CSF, TSLP, VEGF, IP-10, KC, MCP-1, MIP-1a, and RANTES. Differences in median cytokine levels between groups were analyzed using two-tailed unpaired Mann-Whitney U test.
Results: In both CAS- and vehicle-treated rats IL-1a, IL-6, IL-10, IL-18, IFN-γ, TNF-α, TSLP, VEGF, IP-10, KC, MCP-1, MIP-1a and RANTES could be detected in lungs, whereas in serum all cytokines, except IL-1, IL-2, IL-22 and G-GSF could be detected. In the lungs of CAS-treated rats, the anti-inflammatory IL-10 expression was high on day 3 compared to vehicle-treated rats. This was accompanied by higher anti-inflammatory cytokine levels of IL-5, IL-10, and IL-13 in serum of CAS-treated rats compared with vehicle-treated rats on day 3. The expression of IL-10 in CAS-treated rats decreased on day 6.
Low levels of pro-inflammatory cytokines such as IFN-γ were observed in lungs of CAS- and vehicle-treated rats on days 3 and 6. IL-18 was increased in lungs of CAS-treated rats compared to vehicle-treated rats during infection. In serum a higher expression of IL-12p70, IFN-γ, IL-6, IL-18 and IL-1a was observed in CAS-treated rats compared to vehicle-treated rats on day 3. The CC-chemokines in lungs and serum were slightly increased in CAS-treated rats and decreased on day 6.
Conclusion: This study shows that human pharmacokinetic-equivalent CAS treatment does not skew the immune response to an anti- or pro-inflammatory profile during neutropenia in IPA. Antigen exposure is altered after intense cell wall remodelling following exposure of fungal cells to CAS during neutropenia. When neutropenia is resolved, an altered cytokine micro-environment could influence the eventual elimination of the fungus by host phagocytes. The mechanisms involved remain to be investigated.

O088

HEV infection among Dutch blood donors, 1988-2012

H.L. Zaaijer[1,2], E. Slot[2], B.M. Hogema[2]
[1]*AMC, KlinVir, Amsterdam,* [2]*Sanquin, BOI, Amsterdam*

Introduction: In Europe, the dynamics of endemic hepatitis E virus (HEV) infection remain enigmatic. We studied the presence of silent HEV infection among Dutch blood donors.
Methods and results: Using donations collected throughout the country in 2011 and 2012, 40,176 donations were tested for HEV RNA in 459 pools of 48 or 480 donations. Deconstruction of the reactive pools identified 13 viremic donors. In addition, 5239 donors were tested for presence of IgG and IgM anti-HEV; and for HEV RNA when IgM positive. 1401/5239 (27%) donations tested repeat-positive for HEV IgG, of which 49 (3.5%) also tested positive for IgM anti-HEV. Four of the HEV IgM positive donors tested positive for HEV RNA.
HEV IgG seroprevalence in recent donations ranged from 13% among donors younger than 30 years to 43% in donors older than 60. Surprisingly, the HEV IgG seroprevalence in archived Dutch donor samples from 1988 and 2000 was found to be even higher. Analysis of dates-of-birth and seropositivity in the samples from 1988, 2000 and 2011/12 indicates an age-cohort effect. Possibly endemic HEV infection recently returned, after being absent during many years.
Conclusion: The finding of 17 HEV RNA positive donations among 45,415 recent donations corresponds to 1 HEV positive blood donation per day in the Netherlands. For 16 of the 17 HEV RNA positive donors genotyping succeeded, revealing HEV genotypes 3c and 3f, which circulate among Dutch pigs. Apparently, silent HEV infection is common in the Netherlands, which possibly applies to larger parts of Europe.

O089

HEV infection post allogenic hematopoietic stem cell transplantation recipients may be misdiagnosed as graft versus host disease or drug toxicity

S.D. Pas, J. Versluis, E. Agteresch, R.A. de Man, J. Maaskant, A.D.M.E. Osterhaus, J. Cornelissen, A.A. van der Eijk
Erasmus MC, Dept. of Virology, Rotterdam

Hepatitis E virus (HEV) is the causative agent of acute and chronic hepatitis and gastro-enteritis. It is an emerging health issue in industrialized countries, particularly in the immunocompromised. Since little is known of HEV

infections in allogeneic hematopoietic stem cell transplantation recipients (alloHSCT), we studied HEV infection in alloHSCT.

Patients receiving alloHSCT between January 2006-July 2011 were included. Anti-HEV serostatus (Wantai assay) before alloHSCT and presence of HEV RNA after alloHSCT was assessed. Additionally, all plasma samples from episodes characterized by common toxicity criteria grade 2-4 liver function abnormalities were screened for HEV RNA. From confirmed cases, the course of HEV infection and clinical implications were studied retrospectively and phylogenetic analysis was performed.

328 Patients were included in the study whose median age was 50.4 years (range 17-66). 54% were male and 57% of the included recipients were alive at the end of follow up. In total, eight HEV infected cases (2.4%) were identified, of which five developed chronic HEV. These were misdiagnosed before as hepatic Graft versus host disease (GVHD, n = 5), or drug-toxicity (n = 3). HEV-ORF1 sequences classified all cases as genotype 3 and ruled out a common source. Seroprevalence prior to alloHSCT was 12.9%, 2 patients (0.6%) were anti-HEV-IgM positive, though HEV viremia could not be confirmed by PCR. The median time from alloHSCT to infection was 4.6 months (range -2-18 months). Four of eight cases died with HEV viremia, signs of ongoing hepatitis (n = 4) and neurologic disease (n = 1), after a median period of infection of 4.1 (range 2-12) months. The four living patients cleared HEV after a median period of 8.8 (range: 2-42) months, supported by ribavirin treatment (n = 1) and reduction of immune suppression (n = 3). Two of four living patients were diagnosed with chronic hepatitis and fibrosis by liver biopsy. One HEV patient presented with recurring episodes of viremia, characterized as viral reactivation.

Although HEV is a relatively infrequent opportunistic pathogen after alloHSCT, a differential diagnosis including hepatitis E is mandatory given the clinical impact. Future alloHSCT recipients should be screened for HEV RNA prior to transplantation and should be monitored after alloHSCT, especially during episodes of intensive immunosuppressive therapy and if liver abnormalities occur.

O090

Lack of X4-tropic HIV prevents viral rebound post CCR5-fj32 stem cell transplantation in the 'Berlin Patient'

J.J. Symons, S.G. Deeks, G. Hütter, A.M.J. Wensing, J. Martin, P.M. van Ham, L. Vandekerckhove, M. Nijhuis
University Medical Center Utrecht, Dept of Medical Micobiology, Utrecht

Background: The 'Berlin patient' is the first patient functionally cured of HIV. He received stem cell transplantation from a homozygote *CCR5-fj32* donor and has apparently been cured. The reconstituted $CD4^+$ T-cell population should be susceptible to infection with CXCR4-using viruses. According to gp120-V3 deep sequencing analysis of plasma-derived variants present before transplantation, the patient harbored minority (2.9%) viruses which were CXCR4-predicted ($geno2pheno_{coreceptor}$ FPR 10%). It is hence unclear why these failed to emerge post-transplant. We hypothesize that these CXCR4-predicted variants depend on CCR5 for replication.

Methods: Patient-derived viral constructs were generated by cloning V3-sequences of the CXCR4-predicted viruses (pX1-pX7) and the dominant CCR5-predicted strain (pR5) into HXB2- fjV3. As controls V3-sequences of HXB2 (cHXB2; CXCR4-tropic) and BaL (cBaL; CCR5-tropic) were cloned. Co-receptor preference was investigated in U-373-MAGI cells expressing $CD4^+CCR5^+$ or $CD4^+CXCR4^+$, PBMCs from healthy donors and patient-derived post-transplant *CCR5-fj32* PBMCs.

Results: Three pre-transplant CXCR4-predicted strains had an amino acid substitution in the V3 glycosylation-motif and one had a lysine at position 25, associated with CXCR4-tropism. Five of the 7 viral clones were infectious. cHXB2 infected $CD4^+CXCR4^+$-MAGI-cells and was inhibited by AMD-3100 (CXCR4-inhibitor) in donor PBMCs. Remarkably, the CXCR4-predicted viruses (FPR 2.7-9.3) depended on CCR5 for replication in $CD4^+CCR5^+$-MAGI-cells and were inhibited by maraviroc (CCR5-inhibitor) in donor PBMCs similar to pR5 and cBaL. As an ultimate proof it was shown that CXCR4-predicted strains could not replicate in the post-transplant derived *CCR5-fj32* PBMCs, whereas cHXB2 replication was observed.

Conclusion: The minority population of CXCR4-predicted viral strains which the patient harbored pre-transplant were fully dependent on CCR5 for replication *in vitro*. This could explain lack of rebound after treatment discontinuation. This provides a strong rationale for the further development of CCR5-targeted gene therapy and suggests that successful reconstitution of CCR5-depleted immune system may work, even if there is some evidence of CXCR4-predicted variants.

O091

The antipicornaviral activity of the antifungal drug itraconazole

L. van der Linden[1], R. Ulferts[2], K.H.W. Lanke[1], M. Arita[3], P. Leyssen[4], H. Shimizu[3], J. Neyts[4], F.J.M. Van Kuppeveld[2]
[1]NCMLS / N4i, UMC St Radboud, Dept. of Medical Microbiology, Nijmegen, [2]Utrecht University, Virology Division, Dept. of Infectious Diseases, Utrecht, [3]National Institute of Infectious Diseases, Dept. of Virology II, Tokyo, Japan, [4]Rega Institute for Medical Research, University of Leuven, Laboratory of Virology and Chemotherapy, Leuven, Belgium

Currently, no antiviral therapy is available for treatment of infections with enteroviruses. This genus of the family *Picornaviridae* includes many important pathogens such as poliovirus, enterovirus 71, and coxsackievirus.

To identify small-molecule compounds with antiviral activity against enteroviruses, we screened a small-molecule library for inhibitors of coxsackievirus B3 (CVB3)-induced cytopathic effect. In this screen, we found that the antifungal drug itraconazole possessed antiviral activity. Itraconazole displayed broad-spectrum antiviral activity, inhibiting a range of enteroviruses as well as mengovirus, a member of the cardiovirus genus. Using subgenomic replicon studies, we showed that the compound acted at the stage of RNA replication. Mutations in the viral non-structural protein 3A of CVB3 and poliovirus provided resistance against the compound. Remarkably, itraconazole had no effect on the activity of the target of other compounds to which these mutations confer resistance, i.e. phosphatidylinositol-phosphate-4-kinase III bèta (PI4KIII). A further effort to elucidate the mechanism of action of itraconazole, showed that several reported targets of itraconazole do not mediate the antiviral effect of itraconazole, including hCYP51, the human homologue of the fungal target of itraconazole.

In sum, we showed that itraconazole possesses broad-spectrum antiviral activity against picornaviruses, thereby providing an interesting starting point for further studies into itraconazole or related compounds as an antiviral drug. Additionally, in the future, the identification of the relevant target of itraconazole is expected to expand our understanding of picornavirus replication on the one hand, and to benefit antiviral drug development on the other hand.

O092

Association between BK polyomavirus serostatus and post transplantation viremia in a kidney transplant cohort of living related donor-recipient pairs.

H.F. Wunderink, C.S. van der Blij-de Brouwer, P.Z. van der Meijden, J.W. de Fijter, A.C.M. Kroes, A.C.T.M. Vossen, J.I. Rotmans, M.C.W. Feltkamp
Leiden University Medical Center, Dept. of Medical Microbiology, Leiden

Introduction: BK polyomavirus (BKV) is a small (45 nm) double stranded, non-enveloped, DNA virus with a worldwide distribution and is ubiquitously present in the human population. The primary infection mainly occurs in early childhood and is generally asymptomatic. The seroprevalence increases with age to 80-95% in the adult population. After primary infection the virus remains latent in renotubular epithelial cells. In kidney transplant patients the virus can reactivate shown by detectable viremia, and cause polyoma virus associated nephropathy (PVAN). PVAN can cause irreversible loss of graft function or loss of the graft in 1-10% of cases. The goal of this study was to correlate BKV serostatus of donors (D) and recipients (R) with the development of BKV viremia post transplantation. We hypothesized that BKV seronegative recipients from seropositive donors have the highest risk of developing viremia.

Methods: 198 living related donor and recipient pairs from the period between 2007 and 2010 were included in the study. A total of 396 pretransplant serum samples were tested for the presence of BKV VP1-directed antibodies using in-house luminex-based serology. BKV load was measured with RT-PCR in plasma samples taken 1.5, 3 and 6 months after transplantation.

Results: 95% of the donors and 94% of the recipients were BKV seropositive at the time of transplantation. Overall, viremia any time after transplantation was observed in sixty recipients (30%), fifty-four of which were seropositive (90%) and six (10%) seronegative. All viremic recipients had a seropositive donor. Six of twelve (50%) seronegative recipients developed viremia, and 54 of 178 (30%) seropositive recipients. Seropositive recipients from seronegative donors did not show viremia (0/9, 0%). The serostatus of the donor was significantly correlated with the development of viremia (p = 0,047), the serostatus of the recipient was not (p = 0,127)

Conclusion: The overall BKV seroprevalence was high and viremia was frequently observed. Seronegative recipients were more often viremic than the seropositive ones (50 vs. 30%), and therefore seem more at risk of PVAN. In recipients of seronegative donors no viremia was observed, indicating that BKV reactivation originates from the donor kidney and suggesting that the risk of PVAN from such donors is very low. The BKV serostatus of the donor was just significantly correlated with the development of viremia, whereas the recipient serostatus was not. By extending our studies we will further investigate the relation between BKV serostatus and viremia, and determine the usefulness of serological testing prior to transplantation to predict BKV viremia and PVAN risk.

O093

Noise promotion of autorepressed abrB by a small regulatory RNA

R.A.T. Mars[1], P. Nicolas[2], U. Völker[3], U. Mäder[3], J.M. van Dijl[4], E.L. Denham[4]
[1]UMCG/RUG, Dept. of Medical Microbiology /Molecular Bacteriology, Groningen, [2]INRA, Mathématique Informatique et Génome, Jouy-En-Josas, France, [3]Ernst-Moritz-Arndt-University Greifswald, Interfaculty Institute for Genetics and, Greifswald, Germany, [4]University of Groningen, University Medical Center Groningen, Dept. of Medical Microbiology, Groningen

In their natural habitat, bacteria must constantly adapt to changing environments and simultaneously anticipate further disturbances. To do this, intricately interlinked metabolic and genetic regulation has evolved. Small regulatory RNAs (srRNAs) are part of this complex network of regulation and act by fine-tuning mRNA or protein levels to coordinate (stress) responses. Many studies have characterized the short complementary base pairing of srRNAs to mRNA molecules, that act for instance by interacting with the ribosome binding region (RBS) to inhibit translation. However, very few studies have focused specifically on the physiological reason for a specific srRNA-target interaction.

AbrB is a global transcriptional regulator that represses the expression of hundreds of genes in the exponential growth phase of the gram-positive bacterium *Bacillus subtilis*. Transcription of abrB is autorepressed by abrB dimers binding to its own promoter. abrB is also repressed by SpoOA and, more strongly by SpoOA-P, that accumulates when cells enter stationary phase growth. This results in depletion of abrB levels upon entry into stationary phase that consequently leads to depression of abrB repressed genes, which are often important for stationary phase processes. Because of this role in the elaborate sporulation and competence decision-making network abrB has mainly been studied in the context of entry into stationary phase, and less is known about its exact role in the exponential growth phase.

Genes can be expressed with a large variability, with high expression levels in some cells and low expression levels in others. Depending on the function of the protein, this variability in protein levels – or protein expression noise – can be detrimental or beneficial for a cell. Interestingly, particularly noisy genes are often found to be regulators of development and bacterial persistence. Because of the importance of noise, cells have evolved mechanisms to regulate noise levels of at least some proteins. Reducing noise has been suggested as an important explanation why many transcriptional regulators in bacteria (~50% in *E. coli*) repress the transcription of their own promoter (autorepression).

We screened several deletion strains of conserved putative *B. subtilis* srRNAs that are highly expressed on defined minimal medium for growth phenotypes. One mutant displayed increased growth rates on minimal medium with slowly metabolized carbon sources. Inspection of evolutionary conserved predicted targets for this putative srRNA has led us to testing whether this aberrant growth was due to higher abrB levels in these strains. Here we report that, under some growth conditions, *B. subtilis* employs an srRNA to regulate translation – and more specifically to modulate the protein expression noise – of abrB, a protein encoded by a gene with low transcriptional noise because of its autoregulation. This study shows that bacteria can employ srRNAs to specifically fine-tune protein expression noise levels, and reveals the importance of distinguishing between transcriptional noise and translational noise.

O094

A combination of genome-scale analyses and evolutionary engineering of butanol tolerance in *Saccharomyces cerevisiae* reveal an essential role of protein degradation

D. González Ramos[1], M. van den Broek[2], J.A. van Maris[2], T. Pronk[2], G. Daran[3]

[1]TUDelft, Biotechnology. Industrial Microbiology section, Delft, [2]Kluyver Centre for Genomics of Industrial Fermentation, Delft, [3]Platform for Green Synthetic Biology, Delft

Butanol produced from renewable biomass is a promising alternative to ethanol due to its interesting properties as a fuel. *Saccharomyces cerevisiae* has been previously engineered for butanol production, resulting in strains that produce butanol concentrations that can inhibit its growth. Toxicity is one of the main limitations in the fermentative butanol production and increasing the tolerance level of the producing host is essential in order to make it a competitive industrial process. For this reason, a better understanding of the stress response of *S. cerevisiae* to butanol is an important step to engineer it for improved tolerance. By combining a screening of the haploid *S. cerevisiae* knock-out library, gene overexpression, and genome analysis of butanol tolerant evolutionary engineered strains, we identified that protein degradation plays an essential role on butanol tolerance. Strains deleted in genes involved in the ubiquitin-proteasome system and the vacuolar degradation of damaged proteins, showed hyper sensitivity to butanol. The overexpression of YLR224w, encoding the subunit responsible for the recognition of damaged proteins of an ubiquitin ligase complex, resulted in a strain with a higher butanol tolerance. Additionally, two independent butanol tolerant evolutionary engineered strains carried different mutations in the genes RPN4 and RTG1, which encode transcription factors involved in the expression of proteasome and peroxisomal genes, respectively. The introduction of the mutated alleles in the reference strain increased butanol tolerance, confirming their relevance in the higher tolerance phenotype. The evolved strains, in addition to *n*-butanol, were also more tolerant to 2-butanol, isobutanol and 1-propanol, suggesting a common tolerance mechanism to C3 and C4 alcohols. This study shows that maintenance of the protein integrity is an essential function to tolerate butanol stress and a new promising target to engineer *S. cerevisiae* for improved tolerance. The study also provides the first experimental evidence of increasing the tolerance limits in yeast.

O095

Construction and characterization of a synchronized bacterial oscillator

B.M. Ryback, D.I. Odoni, R.G.A. van Heck, M.C. Hesselman, Y.M. van Nuland, V.A.P. Martins dos Santos, M.W.J. van Passel, F. Hugenholtz

Wageningen University, Systems & Synthetic Biology, Wageningen

Introduction: In order for synthetic genetic circuits to be technologically useful and modularly composable in higher order systems, their properties must be subject to formal mathematical descriptions that capture the salient features of a given circuit. The aim is to develop models that are sufficiently accurate and comprehensive to provide a basis for predicting the circuit's dynamics under varying conditions. To this end, synthetic biologists have adopted approaches originating from the traditionally non-biological fields of nonlinear dynamics and systems & control theory. However, due to the multiplicity of complex molecular interactions affecting the emergent properties of biological systems, mechanistic descriptions of even the simplest genetic circuits (transcriptional feedback oscillators, bi-stable switches) produced by these methods tend to be either oversimplified or numerically intractable. More comprehensive and realistic models can be achieved by constructing 'toy' genetic circuits that provide the experimenter with some degree of control over the transcriptional dynamics, and allow for experimental set-ups that generate reliable data reflecting the intracellular biochemical state in real time.

To this end, we have constructed a genetic circuit capable of producing synchronized oscillatory green fluorescent protein (GFP) expression in small populations of *Escherichia coli* cells. Based on refactored quorum sensing genes and regulatory elements, as well as canonical transcriptional repressors, the system produces transient GFP expression the dynamics of which are mainly dependent on cell density and the concentration of independently variable inducer molecule concentrations.

Methods: The genetic circuit was composed of BioBrick standard biological parts and assembled using a hierarchical scheme of restriction digests followed by ligation and transformation.

Combinatorial simulations in which the terms representing cell density and inducer molecule concentrations were iteratively varied were performed in MATLAB using a numerical solver for delay differential equations. For each variable, values were chosen to range from 0 to the theoretical maximum.

The predictions were tested experimentally by measuring the fluorescence of small volumes of high density *E. coli* cultures in 96-well plates over 12 hours. We employed an experimental design whereby a wide variety of conditions were initially tested with few biological replicates in order to narrow down the conditions most conducive to producing dynamic gene expression. These conditions were then tested with more replicates.

Results: Computational simulations based on a dynamic model of the circuit predicted that the introduction of these chemical control elements substantially broaden the range of conditions under which such synchronized systems can oscillate, in addition to allowing the frequency of the oscillation to be tuned. Plate reader experiments confirmed that transient dynamic GFP expression occurred.

Conclusion: While some of the observations were consistent with the simulations, the drawing of statistically powerful, quantitative conclusions was confounded by the system's inherent stochasticity and the lack of experimental throughput. It is our hope that researchers with access to more sophisticated experimental set-ups, such as microfluidic bioreactors or fluorescent cell sorting machines, will employ this versatile genetic circuit in developing and benchmarking data-driven modeling approaches in the future.

O096

Dissection of yeast responses to extreme calorie restriction and energy starvation

P. Daran-Lapujade, L.G.M. Boender, M.J.H. Almering, M. Dijk, A.J.A. van Maris, J.T. Pronk

Delft University of Technology, Dept. of Biotechnology, Delft

Cultivation methods used to investigate microbial calorie restriction often result in carbon and energy starvation. This study aims to dissect cellular responses to calorie restriction and starvation in *Saccharomyces cerevisiae* by using retentostat cultivation.[1] In retentostats, cells are continuously supplied with a small, constant carbon and energy supply, sufficient for maintenance of cellular viability and integrity but insufficient for growth.[2,3] When glucose-limited retentostats cultivated under extreme calorie restriction were subjected to glucose starvation, calorie-restricted and glucose-starved cells were found to share characteristics such as increased heat-shock tolerance and expression of quiescence-related genes. However, they also displayed strikingly different features. While calorie-restricted yeast cultures remained metabolically active and viable for prolonged periods of time, glucose starvation resulted in rapid consumption of reserve carbohydrates, population heterogeneity due to appearance of senescent cells and, ultimately, loss of viability. Moreover, during starvation, calculated rates of ATP synthesis from storage carbohydrates were 2-3 orders of magnitude lower than steady-state ATP-turnover rates calculated under extreme calorie restriction in retentostats. Stringent reduction of ATP turnover during glucose starvation was accompanied

by a strong down-regulation of genes involved in protein synthesis. These results demonstrate that extreme calorie restriction and carbon starvation represent different physiological states in *S. cerevisiae*.
This work was supported by the Kluyver Center for the Genomics of Industrial Fermentations.

References

1. Boender LG, et al. Extreme calorie restriction and energy source starvation in Saccharomyces cerevisiae represent distinct physiological states. Biochim.Biophys. Acta. 2011;1813:2133-2144.
2. Boender LG, et al. Cellular responses of Saccharomyces cerevisiae at near-zero growth rates: transcriptome analysis of anaerobic retentostat cultures. FEMS Yeast Res. 2011;11:603-620.
3. Boender LG, et al. Quantitative physiology of Saccharomyces cerevisiae at near-zero specific growth rates. Appl. Environ. Microbiol. 2009;75:5607-5614.

O097

Genome mining of the rhizosphere bacterium *Pseudomonas* sp. SH-C52

M. van der Voort
Wageningen University, Laboratory of Phytopathology, Wageningen

Soil ecosystems represent an enormous untapped resource for discovering novel micro-organisms, traits and bioactive genes. Natural disease suppressive soils are particularly interesting as they have a relatively higher abundance of beneficial micro-organisms that guard plants against infections by soil-borne pathogens. By using both culture-independent and culture-dependent approaches, we discovered a novel group of *Pseudomonas* species in the rhizosphere of sugar beet seedlings grown in a soil that is suppressive to the fungal pathogen *Rhizoctonia solani*. Representative strain *Pseudomonas* sp. SH-C52 was shown to inhibit hyphal growth of *R. solani* and various other fungal and oomycete pathogens of plants and fish. Phylogenetic studies showed this strain is closely related to *Pseudomonas corrugata* strains. Sequencing of *Pseudomonas* sp. SH-C52 revealed a genome size of 6.7 Mb with approximately 4% of the genome dedicated to secondary metabolism. *In silico* analysis showed the presence of six large nonribosomal peptide synthetase (NRPS) gene clusters. The first NRPS cluster was predicted to encode for a 9-amino acid chlorinated lipopeptide, designated thanamycin. The partial structure of thanamycin, resolved by nanoDESI mass spectrometry, was consistent with the predicted structure. The antifungal activity of thanamycin was confirmed by mutagenesis of the biosynthesis genes and by in vitro bioassays with the purified compound. Activity of *Pseudomonas* sp. SH-C52 against oomycete pathogens was shown to be mainly due to a second NRPS-encoded peptide, which was predicted to consist of a 22-amino acid peptide moiety. The third lipopeptide, designated brabantamide, is a 2-amino acid peptide linked to a C14-glycosylated fatty acid. Its' biosynthesis is governed by a 12-kb cluster of genes encoding an NRPS, a glycosyltransferase, a monooxygenase, a transcriptional regulator and an RND-type efflux protein. Activity assays showed that this dipeptide has broad-spectrum antibacterial activities. The three other NRPS gene clusters, along with other secondary metabolites, produced by *Pseudomonas* sp. SH-C52 are subject of ongoing genetic and biochemical studies.
In conclusion, this study provides new insights into the diverse secondary metabolism of rhizosphere bacteria and their role in natural disease suppressive soils.

O098

Stringent response activation via phosphate stress modulates *Mycobacterium tuberculosis* capsular components a-glucan and arabinomannan

R. van de Weerd[1], J. Maaskant[1], E. Dainese[2], R. Manganelli[2], C.M.J.E. Vandenbroucke-Grauls[1], W. Bitter[1], B.J. Appelmelk[1], J.J. Geurtsen[1]
[1]VU Medical Center, Dept. of MMI, Amsterdam, [2]University of Padua, Histology, Dept. of Microbiology, Padua, Italy

Mycobacterium tuberculosis, the causative agent of tuberculosis, claims more than 1.4 million lives each year. The tubercle bacillus expresses a wide arsenal of immunomodulatory factors for successful colonization in its host. Most of these factors are outermost surface components of the cell envelope including capsular components. The presence of the capsule was already suggested many decades ago but it was not until recently that our group, for the first time, was able to visualize this layer in a close to native state. The mycobacterial capsule, a loosely attached layer mainly composed of proteins and polysaccharides, may be important for the survival of the bacillus in the host. Although the chemical composition of the capsule is relatively well understood, questions concerning biosynthesis, transport, regulation and biological relevance are still open. Virtually, no data is available on how the mycobacterial capsule is affected by stress conditions *in vivo*.
The aim of this study was to unravel mechanisms involved in mycobacterial capsule biosynthesis regulation, under exogenous stress. We studied the main capsular component a-glucan under inorganic phosphate (Pi) stress and by direct stringent response (SR) activation, an essential stress response pathway activated in *M. tuberculosis in vivo*.
First, we used a transposon mutagenesis screen in the model organism *M. smegmatis* to identify mutants with altered a-glucan production. From this screen, we picked up a group of strongly induced a-glucan mutants with mutations in the ABC phosphate transport locus Pst. The Pst system is also important in Pi acquisition in *M. tuberculosis*, so we hypothesized that capsule production is elevated when *M. tuberculosis* experiences Pi stress. We confirmed our ideas when we challenged the bacillus by limited Pi availability. Furthermore, we identified sigma factor E (SigE) as essential for the Pi stress related up-regulation of capsular a-glucan, by using targeted knockouts of the sigE gene. Finally, we have identified that the SR pathway is required for regulation of mycobacterial capsular polysaccharides a-glucan and arabinomannan, by using serine hydroxamate, the chemical activator of the SR. By further analysis of capsule modulation under stress conditions both *in vitro* and *in vivo*, we aim to unravel the mysteries of the biological function of the capsule and its possible contribution to persistence. These insights will not only increase our knowledge about the role of the capsule during infection, but they may lead to define important TB drug targets for intervention.

O100

Computer assisted teaching in the UK and the US: *@10queues – #adaptordie*

W.P. Duprex
Boston University School of Medicine, Dept. of Microbiology and National Emerging Infectious Diseases Laboratories (NEIDL), Boston, USA

#sgmdub12: Someone from my UK laboratory laughed and shot a look of disbelief, tinged with pity when, just before the *Viral Zoonosis* symposium was about to begin at the Society for General Microbiology annual meeting, I asked the simple question, "what's a hashtag?" To him this seemed tantamount to asking, "is Dublin where Guinness comes from?" or "what's the point in vaccinating my kid?" Therefore if you, like me at the time I asked that question, need a translation of the above title, then this presentation is for you.
As college educators in Europe or in the United States there is one certainty, our students will always be getting younger. Indeed this is a comment which is oftentimes heard each October when professors discuss the new student intake. As microbiologists our desire should be to motivate the next generation of scientists and to ensure the best young minds enter our discipline. If that is our goal then one thing is certain, we need to recognize that our students learn and interact in ways that are, most likely, far from familiar to us. In part this is due to the very different approaches used in elementary, middle and high schools compared to those used when we were taught. However, that is not the only major difference. Social media, fast and furious flow of bite-sized bytes of information and unprecedented international interconnectivity might not make the world a smaller place but certainly makes it a fundamentally different place. As microbiologists I argue that our inherent understanding of the, #adaptordie principle, which every organism under selective pressure faces, should position us to adopt novel teaching methods and be developmental in how we interact with our students. In doing so we will empower these individuals to perform experiments using state of the art tools and techniques move thereby moving our discipline into new and exciting territories.
#KNVM12: It is clear that contemporary approaches are vital in a world where there are ever increasing pressures on scientists. All too often we are not trusted by non-scientists based on weak communication skills and are much less developmental than we should be in how we share our knowledge. Whether we have taught for three or thirty years it is probably fair to say that most likely the majority of us choose not to embrace social media-based approaches as educators and scientists. Nevertheless, we have an obligation to our students and also the general public, whose taxes oftentimes fund the studies we perform, to make our science both relevant and accessible. Thus, in this lecture I will chart how, after discovering the power of a hashtag @10queues hopped into uncharted waters and outline how this discovery has comes full circle and is influencing ***#ESV13*** and ***#sgmliv14***.

O101

App-lication of tablets in teaching

N.W. Boot
Leiden University of Applied Science, Techniek, HLO, Leiden

The application of tablets, or app-based devices, as a tool is currently one of the most discussed topics in teaching. Tablets give both access to internet-based ways of teaching as well as dedicated app-based teaching. In this short talk I will discuss the ways tablets are currently used in teaching and the ways they might be used as a powerfull tool in scientific teaching in the (near) future.

O102

Apps for labs

J. van der Kolk, R.J.M. Hartog, H. Gruppen
Wageningen University, Laboratory of Food Chemistry, Wageningen

Laboratory classes play an important role in science education. During a laboratory class, students are considered to obtain manipulative skills, learn to observe, learn to plan experiments, learn to deal with (often ambiguous) results, etc. Four issues with laboratory classes

have been identified. Firstly, students often have to deal with extraneous cognitive load caused by the instructional format of the laboratory class. Secondly, students often do not prepare for their laboratory class. Thirdly, the communication between supervisors and students can be suboptimal, especially in larger-scale laboratory classes. Fourthly, supervisors might fear a loss of control during inquiry based laboratory classes.To address these issues, a laboratory electronic performance support system (labEPSS) was designed, realized, used and evaluated at the Laboratory of Food Chemistry of Wageningen University. This labEPSS consists of the following tools:
A web-based laboratory manual, aiming to provide students with just-in-time procedural information (e.g. how an apparatus looks like, where chemicals can be found).
A web-based experiment design tool, aiming to let students design their research strategy as a workflow beforehand and support students while carrying out this strategy in the laboratory.
A web-app' for students' smartphones providing the same functionalities as the digital laboratory manual.
A web-based equipment booking system, which is part of the web-based experiment design tool.
Based on the evaluations it was concluded that students and supervisors appreciated labEPSS and that labEPSS is capable of addressing above issues. Finally, an overall re-design of labEPSS is proposed, in which the tools offer an integrated experience. Because labEPSS is highly configurable, it can be used in different laboratory classes throughout various curricula.

O103
Genome evolution in a long-term experiment with *Escherichia coli*

D. Schneider
Laboratoire Adaptation et Pathogénie des Micro-organismes, Université Joseph Fourier, Grenoble, France

Evolution is inherently complex owing to the high dimensionality of genomes and the multitude of interactions between genes, gene products, metabolites, and environmental factors including resources. Historically, most studies have focused on components or subsets of complex biological systems. However, to understand many adaptations, one must investigate changes in entire genomes and examine their consequences for global expression and organismal performance. To achieve this goal, the molecular-genetic and phenotypic bases of adaptation are investigated in a bacterial model where twelve independent populations have been propagated in the same environment from a common *Escherichia coli* ancestor for 50,000 generations. All populations achieved substantial fitness improvement during evolutionary time. Adaptive changes have been shown to be associated with complex effects on global gene expression, including widespread pleiotropy and epistasis, indicative of important changes in regulatory networks. In addition to their expression, bacterial genomes revealed an exceptional dynamics in their mutation rates, reflecting a tension between adaptation and genetic load that is strongly related to the fit of the bacterial populations to their environment. This evolution experiment addresses how evolvable are genomic features and the molecular bases of the evolvability of networks.

O105
Evolutionary adaptation of *Akkermansia* species within the mammalian host

J.P. Ouwerkerk[1], C. Belzer[1], W.M. de Vos[1,2]
[1]WUR, Laboratory of Microbiology, Wageningen, [2]University of Helsinki, Dept. of Basic Veterinary Medicine, Division of Microbiology and Epidemiology, Helsinki, Finland

Akkermansia muciniphila is an abundant member of the healthy human intestine, and has its habitat in the mucin layer that lines the gut. The microbe is seen as beneficial as its abundance is inversely correlated with IBD, appendicitis, autism and obesity. *Akkermansia* like sequences are universally distributed among the intestines of animals ranging from mammals, domesticated and wild, to non-mammals, such as chicken, zebrafish and Burmese pythons. This broad range of hosts differ in their GI-tract anatomy and diet, suggesting adaptation of *Akkermansia* spp. to host physiology. Furthermore colonisation of this broad range of hosts indicates that there could be a specific functionality for *Akkermansia* spp. within the gut microbiota.
The aim of this study is to isolate and compare new *Akkermansia* spp. to obtain insights into the adaptation and functionality of *Akkermansia* spp. within the GI-tract.
A phylogenetic tree was generated, indicating the position of the type strain *Akkermansia muciniphila* among selected full-length 16S rRNA clones from mammalian gut samples.
Faecal samples from 50 mammals were collected and tested for the presence of *Akkermansia* spp. or other Verrucomicrobia. New *Akkermansia* strains were isolated, by using mucin as only carbon and nitrogen source. The full-length 16S rRNA gene was sequenced and DNA fingerprinting was used to make a genomic comparison. Physiology of the isolates was determined by testing the metabolic capacity en SCFA production.
The phylogenetic comparison of *Akkermansia*-related 16S rRNA gene sequences indicate the presence of five clades of which four contain sequences associated with human gut samples. The sequence similarity between the type strain of *A. muciniphila* and other sequences within these four clades ranges from 80 to 100%. This suggests that colonization of the mammalian gut with different *Akkermansia* species and Verrucomicrobia genera is possible.
Thus far, 19 different pure cultures are obtained from mammalian faecal samples, including 6 human isolates. The 16S rRNA genes show high identity to the type strain. However, the genomic comparison using DNA fingerprinting shows at least 6 different genomic lineages. On top of this, the physiology of the pure isolates shows a high diversity. This indicates genomic adaptation divergent from 16S rRNA gene evolution.
Future genetic characterisations of the new mammalian isolates will verify adaptation of *Akkermansia* spp. to their host. Simultaneously, this genetic characterisation can unravel if there is a specific functionality for *Akkermansia* spp. within the mammalian gut.

O106
Experimental evolution in traditional fermented products

S.E. Schoustra[1], J. Shindano[2], A.J. Poulain[3]
[1]Wageningen University, Genetics, Wageningen, [2]University of Zambia, Food Science and Technology, Lusaka, Zambia, [3]University of Ottawa, Biology, Ottawa, Canada

Introduction: In my work I use experimental evolution to ask how organisms adapt to novel conditions, both as single genotypes and as mixed communities. At this conference, I will present results on the evolution of microbial communities in traditional fermented products from Zambia. Analogous to laboratory experimental evolution, these products are produced by transferring a fraction of a former batch to initiate a new batch of product. In this way, microbial communities are allowed to evolve of large numbers of generations.
Methods: I have sampled three different traditional products (based on either milk or maize) from across Zambia, obtaining a total of 36 samples. I have established the community composition of the products and compared this composition for different samples of the same product type and different product types. In the laboratory, we have propagated the microbial communities for an additional two months under various conditions and have monitored changes in community composition.
Results: Our results show that the microbial flora is dominated by around 6 species of lactic acid bacteria and that specific combinations of species result into stable communities. I show that both geography and anthropogenic factors affect community composition in products collected from Zambia. Laboratory experiments propagating communities over two months show specific shifts in community structure that is consistent over all nine independent communities.
Conclusion: Traditional fermented products are very powerful experimental systems to study long-term properties of entire microbial communities. From the field sampling study we conclude that both environmental factors (that differ by geography) and anthropogenic factors are key in shaping microbial communities. From the laboratory studies we conclude that these communities are very stable in the long-term. This work opens up a range of possible follow-up projects. We will perform manipulative experiments in the field as well as in the laboratory to establish exactly what factors are essential for community stability.

O107
Staphylococcus aureus biofilm matrix does not hide the bacteria, but instead strongly activates the innate immune system

R. Nijland, J.A.G. van Strijp
UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: We study *S. aureus* biofilms and their interaction with the human innate immune system. Although some *S. aureus* infections like sepsis could be regarded as planktonic, most infections like osteomyelitis, endocarditis, or prosthetic implant infections are biofilm-related.[1] It is thought that the biofilm phenotype benefits bacteria by providing a hiding place from the immune system. The biofilm is held together by a hydrated matrix of biopolymers, consisting of (amyloid) proteins, polysaccharides such as PNAG, and extracellular DNA (eDNA). To test if the biofilm is indeed such a good hiding place we studied the effect of the individual biofilm matrix materials on the innate immune system. Amyloids formed from small secreted peptides called Phenol Soluble Modulins (PSMs) play an important role in the protein part of this matrix.[2] We have shown that these peptides have a very strong immune stimulating function, but that this is neutralized by the serum lipoproteins. To determine the contribution of the other biofilm matrix components we tested their effects on the immune system as well.
Methods: We obtained purified PNAG and tested this in an ELISA for activation of complement through the classical, lectin and alternative pathways. Using specific antibodies and (confocal) microscopy we studied the location of the polysaccharides in the biofilm. eDNA was isolated from both static and flow cell grown *S. aureus* biofilms by digesting the biofilm matrix with PNGaseF or DispersinB, which both disrupt the polysaccharides and as such released the other matrix components from the biofilm. This crude eDNA was analysed for non-DNA content, and also tested in an complement ELISA. Biofilm was imaged by time-lapse microscopy to study the interaction with neutrophils.

Results and conclusion: Biofilm amlyoids consisting of PSMs activate neutrophils at very low concentrations. The biofilm polysaccharide PNAG activates complement, mainly through the alternative pathway. Also *S. aureus* eDNA coated on the bottom of a 96 well plate activated the complement system, again through the alternative pathway. Surprisingly, the biofilm matrix components are activating complement instead of cloaking the bacteria. This activation results both in opsonisation of the biofilm and release of anaphylatoxins that will attract neutrophils toward the biofilm. Since in the human body biofilm formation is beneficial for the bacteria, the activation of the complement system and recruitment of neutrophils must interact with the biofilm in such a way that it benefits the bacteria. Using confocal microscopy it becomes clear that the biofilm is impenetrable to antibodies detecting the PNAG, and as such, likely to be impenetrable to the much larger components of the complement system, let alone neutrophils. The recruited innate immune system might form an additional protective layer surrounding the biofilm. We hope to elucidate this intricate and complex interaction of the biofilm with the innate immune system, and how this benefits the bacteria.

References

1. Hall-Stoodley L, et al. Cell Microbiol. 2009;11:1034-1043.
2. Schwartz K, et al. PLoS Pathog. 2012;8:e1002744.

O108

AtlAEfm: the major autolysin in *Enterococcus faecium* involved in cell separation, surface Acm exposure, eDNA release and biofilm formation

F.L.P.l. Paganelli, R.J.L. Willems, P.J. Jansen, X. Zhang, M.J.M.B. Bonten, H.L.L. Leavis

UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Enterococcus faecium developed into a multi-drug resistant nosocomial pathogen during the past two decades and frequently causes biofilm-mediated infections associated with implanted medical devices. Insights into the pathogenesis of biofilm formation in this species are pivotal for the development of new strategies to prevent biofilm formation. In several other species, extracellular DNA (eDNA) is an essential matrix component and contributes to biofilm attachment and stability. Moreover, eDNA is released from bacterial cells that are vulnerable to the action of autolysin which is secreted in the biofilm. In this study, we reveal the contribution of eDNA to biofilm formation and identified and functionally characterized the major autolysin of *E. faecium* E1162. The role of eDNA in biofilm formation was analyzed in a semi-static biofilm model. Biofilm formation of wildtype was decreased by 90% when DNAse was added at T = 0. Using bioinformatics (based on gene annotation), 6 putative E1162 autolysin genes were predicted, and single crossover mutants were constructed. Insertional disruption of only the gene locus tag EfmE1162_2692 showed a reduced eDNA release, deficient cell attachment, decreased biofilm, lysis resistance, decreased cell wall hydrolysis and significant chaining compared to wildtype. This locus, present in all sequenced *E. faecium* strains, was considered the major autolysin and renamed to $AtlA_{Efm}$. $AtlA_{Efm}$ cell surface localization in single cells and biofilm was analyzed by confocal microscopy. Analysis revealed that, in the wildtype, $AtlA_{Efm}$ is present in the cell septum during planktonic log phase, whereas in the biofilm it is secreted in the matrix, indicating that $AtlA_{Efm}$ itself plays a role in matrix stability, apart from eDNA release. Moreover, the $AtlA_{Efm}$ deficient mutant showed reduced binding to collagen type I and IV. Since purified $AtlA_{Efm}$ did not bind to collagen type I and IV, the altered binding phenotype of the mutant was assumed to be mediated indirectly by altered exposition of Acm, an adhesin known to specifically bind to these collagens and considered a virulence factor in *E. faecium*. Confocal microscopy confirmed that in the wildtype Acm is localized at the cell septum and poles, while in the $AtlA_{Efm}$ deficient mutant, because of chaining, only at the septum. Incubation of the $AtlA_{Efm}$ deficient mutant with DNase abrogated chaining and restored exposure of Acm at the poles, and binding to collagen. In conclusion, $AtlA_{Efm}$ is the major autolysin in *E. faecium* and contributes to biofilm stability and Acm cell surface localization, making $AtlA_{Efm}$ a promising target for treatment of *E. faecium* biofilm-mediated infections.

O109

The papain inhibitor (SPI) of *Streptomyces mobaraensis* inhibits bacterial cysteine proteases and is an antagonist of bacterial growth

W.E. Kaman[1], S. Zindel[2], S. Fröls[3], F. Pfeifer[3], A. Peters[2], J.P. Hays[1], H.L. Fuchsbauer[2]

[1]*Erasmus Medical Center, Medical Microbiology and Infectious Diseases, Rotterdam,* [2]*University of Applied Sciences of Darmstadt, Dept. of Chemical Engineering and B, Darmstadt, Germany,* [3]*Technische Universität Darmstadt, Dept. of Biology, Darmstadt, Germany*

Recently, we characterized a novel protein (*Streptomyces* papain inhibitor; SPI) from *Streptomyces mobaraensis* that inactivates cysteine proteases and has the potential to be used as an antimicrobial agent that inhibits the destructive action of cysteine protease virulence factors from pathogenic bacteria. Using a FRET-based peptide probe assay an inhibitory effect of SPI on bacterial cysteine protease activity (*Staphylococcus aureus* SspB), and on bacterial protease-containing culture supernatants (*Porphyromonas gingivalis*, *Bacillus anthracis*) was measured. Further, the growth of the cysteine protease-producing pathogens *Bacillus anthracis*, *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Vibrio cholerae*) was completely inhibited at a concentration of 10 M of SPI during a 12 hour incubation cycle. At this concentration no cytotoxicity of SPI was determined on RAW264.7 cells. We conclude that SPI inhibits the action of prokaryotic cysteine protease virulence factors and has the potential to become a novel therapeutic treatment against a range of pathogenic bacteria.

O110

The ESX-5 secretion system is essential for *Mycobacterium* marinum viability because it controls the permeability of the mycobacterial outer membrane

L.S. Ates, R. Ummels, A.H. Horeman, W.B. Bitter, E.N.G. Houben

VU University Amsterdam, Amsterdam

Mycobacteria are the causative agent of several serious diseases, such as tuberculosis and leprosy. The success of these pathogens depends on the secretion of proteinaceous virulence factors that manipulate and damage the host immune system. To be effective, these virulence factors need to cross the mycobacterial cell envelope that contains besides an inner membrane a unique outer membrane. To be able to transport proteins across this highly impermeable cell envelope, mycobacteria use specialised secretion systems, the type VII secretion systems, of which pathogenic mycobacteria have five: ESX-1 to ESX-5. ESX-5 is the most recently evolved type VII secretion system and is only present in slow-growing mycobacterial species, which include all major pathogens. To investigate the role of individual ESX-5 genes in virulence, we attempted to delete several ESX-5 genes of *Mycobacterium marinum* by allelic exchange using a specialised transducing mycobacteriophage. However, these efforts only succeeded when an additional copy was introduced *in trans*, suggesting these genes are essential for growth. We could unequivocally show their essentiality, by removing the *trans* copy by allelic exchange, which only succeeded when the endogenous copy was still present on the genome. This essentiality of almost all ESX-5 genes of *M. marinum* was also shown by transposon directed insertion site sequencing (TraDIS), a saturated transposon mutagenesis screen combined with next-generation sequencing. To subsequently investigate the mechanism behind the essentiality of the ESX-5 secretion system, transposon mutagenesis was performed to identify mutants, in which esx-5 genes could be deleted. Two transposon mutants were found in this screen, which contained transposons in two different cell envelope lipid biosynthesis genes, previously reported to be involved in cell envelope integrity. Because these mutations seem to point in the direction of cell wall permeability, we also determined the effect of introducing a heterologous pore. These experiments showed that the essentiality of ESX-5 could indeed be alleviated by introducing this gene. Increased outer membrane permeability of the various strains was confirmed by antibiotic disc diffusion assays. Finally, the *M. marinum* strain with the more permeable outer membrane was exposed to transposon mutagenesis and screened for defective secretion of known ESX-5 substrates using a double filter assay. Using this altered strain we could now identify mutants with transposon insertions in several ESX-5 genes that were essential in the wildtype strain according to TraDIS data.

In conclusion, these data show that the essentiality of ESX-5 is linked to the permeability of the mycobacterial outer membrane. Future research will focus on identifying the ESX-5 substrates involved in this process. These factors are attractive targets for new drug and vaccine development to combat mycobacterial diseases.

O111

Disulfide bond formation proteins are essential for complement-resistance of *Moraxella catarrhalis*

S.P.W. de Vries, R. Rademakers, M. Eleveld, C.E. van der Gaast-de Jongh, P.W.M. Hermans, H.J. Bootsma

Radboud University Medical Centre, Pediatric Infectious Diseases, Nijmegen

Background: Over the past years, *Moraxella catarrhalis* has established its position as a causative agent of respiratory tract infections in humans, being a common cause of childhood otitis media (OM) and exacerbations of chronic obstructive pulmonary disease in adults (COPD). The complement system is an important element of the innate immune system and is effective in killing of *M. catarrhalis*. Importantly, most *M. catarrhalis* isolates derived from OM or COPD patients are able to resist complement-mediated killing, and this ability may be considered as an important virulence mechanism of *M. catarrhalis*. In this study, we have used the genome-wide negative selection screenings technology Tn-seq to gain more insight in the molecular basis of *M. catarrhalis* complement-resistance. We identified the dsbB gene, encoding the disulfide bond formation protein B, as being essential for complement-resistance of *M. catarrhalis*. Subsequently, we studied the importance of the disulfide bond formation (DSB) system for complement resistance of *M. catarrhalis* in detail.

Methods: A large *mariner T7* transposon mutant library (~28,000 mutants) of the complement-resistant *M. catarrhalis* strain BBH18 was challenged with NHS or heat-inactivated NHS (control). Negatively selected mutants

with increased susceptibility to NHS were identified by profiling the relative abundance of each mutant using Tn-seq (Transposon insertion site sequencing). For validation of the Tn-seq screen and follow-up, directed gene deletion mutants were generated by allelic exchange and tested for their complement-sensitivity. Genetic complementation of DSB-system mutants was done by expressing the coding sequence from the pSV001 complementation plasmid. Activity of the classical complement pathway was blocked through addition of EGTA and by depletion of IgG. Membrane stability was assessed by incubation of strains in the presence of EDTA.

Results: Our genome-wide screen identified 52 genes that appeared to play a role in complement resistance of *M. catarrhalis*, as their corresponding transposon mutants were negatively selected from the library during serum exposure. Among others, we identified the dsbB gene as being required for complement-resistance of *M. catarrhalis*. We confirmed the essentiality of dsb*B* for complement-resistance in multiple *M. catarrhalis* isolates. Complement-resistance was completely restored to wild-type levels after genetic complementation of O35E fjdsbB. The dsbB protein catalyzes reoxidation of dsbA allowing it to form disulfide bonds in protein. The dsbA gene was not identified in our screen as being essential for complement-resistance as dsbA transposon insertion mutants were not represented in the BBH18 mutant library. Importantly, directed dsbA deletion from strains BBH18 and O35E resulted in loss of complement-resistance, with dsbA mutant strains showing a more severely altered phenotype compared to their corresponding dsbB mutants. Killing of both DSB-system mutants was almost completely abolished after chemical blocking of the classical complement pathway and after depletion of IgG. Interestingly, dsbA mutant strains showed a clear loss of membrane stability.

Conclusion: In this study, Tn-seq was used to examine the molecular basis of *M. catarrhalis* complement-resistance. One of the identified targets, DSB, was shown to play a critical role in evasion of IgG-dependent complement-mediated killing, and is probably required to maintain membrane stability.

O112

The role of staphylococcal PSMs in survival and killing within neutrophils

B.G.J. Surewaard[1], C.J.C. de Haas[1], F.R. DeLeo[2], M. Otto[2], J.A.G. van Strijp[1], R. Nijland[1]

[1]*UMCU, Medical Microbiology, Utrecht,* [2]*NIAID, NIH, Bacterial pathogenesis, Hamilton, Montana, USA*

Introduction: *Staphylococcus aureus* is a major cause of severe infections ranging from mild skin and wound infections to life-threatening disseminated infections. The virulence of *Staphylococcus aureus* is mediated by a number of toxins and immune evasion molecules, including the recently discovered phenol soluble modulins (PSMs). Especially, PSM expression is associated with highly pathogenic community-associated MRSA strains. Two major functions have been addressed to PSMs; they are cytolytic for human neutrophils at micromolar concentrations and chemoattractive at nanomolar concentrations. Hypothesized is that these two functions complement each other; PSMs are actively produced to attract neutrophils to the site of infection and subsequently lyse them. Recently, we have shown that serum inhibits the function of PSMs and that intracellular killing of neutrophils might be the most physiological relevant function.

Methods: Human leukocytes were isolated from peripheral venous blood of healthy donors using a Ficoll/Histopaque gradient. We have investigated the expression of PSMs inside neutrophils by PSM-GFP fusion reporter strains. Isogenic PSM deletion mutants in MW2 background, were labelled with GFP, and CFP to track the location and outgrowth of the bacteria after phagocytosis. Furthermore we analysed the lysis of neutrophils by time-lapse video microscopy and LDH release.

Results: Here, we show that PSMs have a role in killing neutrophils after phagocytosis. By confocal microscopy we show a specific up-regulation of the alpha PSM operon after neutrophil uptake. In addition, PSM knockout strains have reduced capability of lysing neutrophils after phagocytosis, shown by time lapse confocal microscopy and LDH release assays. Furthermore, complementation of the alpha-PSM operon restores the wild-type phenotype, indicating that a-type PSMs are the main staphylococcal intra-cellular killing molecules.

Conclusion: Collectively, our data show that in a serum environment stapylococcal PSMs mainly function as intracellular toxins

O113 - Presented by Thierry Naas

Molecular genetics, epidemiology and biochemistry of emerging antibiotic resistance mechanisms

L. Dortet

Service de Bactériologie-Virologie, Hôpital de Bicêtre & Hôpitaux de Paris, Faculté de Médecine Paris Sud, France

The current worldwide emergence of resistance to the powerful antibiotic carbapenem in *Enterobacteriaceae* constitutes an important growing public health threat. Sporadic outbreaks or endemic situations with enterobacterial isolates not susceptible to carbapenems are now reported not only in hospital settings but also in the community. In *Enterobacteriaceae*, carbapenem resistance arises from two main mechanisms: (1) acquisition of carbapenemase genes that encode for enzymes capable of hydrolyzing carbapenems, or (2) a decrease in the uptake of antibiotics by a qualitative or/and quantitative deficiency of porin expression in association with overexpression of bèta-lactamases that possess very weak affinity for carbapenems.

The most relevant carbapenemases are categorized as three types of enzymes according to the Ambler classification: (1) the KPC-type enzymes (Ambler class A) first described in the US but now found worldwide, (2) the VIM, IMP and NDM metallo-bèta-lactamases (Ambler class B), and (3) the OXA-48-type enzymes (Ambler class D) circulating among Mediterranean countries and progressively disseminating to other geographical areas. KPC enzymes are currently the most clinically-significant enzymes among class A bèta-lactamases. Of note, one specific *K. pneumoniae* clone (ST258) expressing the bla_{KPC} gene has been extensively identified worldwide, indicating that it may have contributed significantly to the spread of the bla_{KPC} genes that are always associated with a given genetic element (transposon Tn4401). Metallo-bèta-lactamases (MBLs) exhibit a broad spectrum of hydrolytic activity including carbapenems, with the exception of aztreonam. Their activity is dependent on the interaction of the bèta-lactamases with Zn^{2+} ion(s) in the active site, explaining the inhibition of their activity by chelators of divalent cations (EDTA). MBLs of IMP and VIM types have spread worldwide since their first description. Their respective encoding genes are usually part of a class I integron which is itself plasmid-encoded. Since it first description 2010, NDM-1 has extensively disseminated worldwide mostly from its Indian reservoir. The bla_{NDM-1} gene is not associated with a single clone, a single species, or to a specific plasmid backbone but has been identified from unrelated gram-negatives, including mostly enterobacterial species, harbored by different plasmid types. Carbapenem-hydrolyzing class D bèta-lactamases (OXA-48, OXA-181) do not hydrolyse expanded-spectrum cephalosporins and possess a weak carbapenemase activity that is not inhibited by either clavulanic acid or by EDTA. A single self-conjugative Inc L/M plasmid of ca. 62-kb is the main source of the bla_{OXA-48} gene dissemination in a variety of enterobacterial species.

The real prevalence of carbapenemase producing bacterial strains remains unknown because many countries worldwide do not report rates of antibiotic susceptibility. The early identification of carbapenemase-producing strains both in clinical infections and/or at the carriage state is mandatory to prevent the development of difficult- or impossible-to-treat infections. Several novel approaches were recently developed for the rapid detection of carbapenemase producers, including spectrophotometry-based approaches and biochemical-based tests (Carba NP test). Implementation of those techniques will help to prevent dissemination and outbreaks involving carbapenemase-producing strains.

O114

A next generation mass spectrometry platform for the rapid identification of (multi-)drug resistant gram-negative bacteria

P.J. Hensbergen[1], F. Fleurbaaij[1], A.A.M. Heemskerk[1], O. Klychnikov[1], S. Paltansing[2], A.M. Deelder[1], O.A. Mayboroda[1], E.J. Kuijper[2], H.C. van Leeuwen[2]

[1]*Leiden University Medical Center, Biomolecular Mass Spectrometry Unit, Leiden,* [2]*Leiden University Medical Center, Dept. of Medical Microbiology, Leiden*

Mass spectrometry (MS) based technologies have recently been introduced for bacterial typing in the clinical microbiology practice. Since 2009, matrix-assisted laser desorption ionization – time of flight (MALDI-ToF) MS based instruments have been implemented in over 10 microbiological laboratories in the Netherlands. Its ability to analyze whole bacterial cells with virtually no sample preparation has drastically improved the time needed to identify a positive culture, starting with a colony. The current generation of MALDI-TOF based diagnostic systems, which detect highly abundant (often ribosomal) proteins, however, are inadequate for detection and identification of subtle protein differences in complex samples. As such, they will in general be insufficient for straightforward detection of proteins involved in antibiotic resistance which are the result of limited proteomic changes, for example due to the expression of antibiotic degrading, modifying or exporting proteins.

In my presentation, I will give an overview of mass spectrometry-based technologies related to clinical microbiology practice with the emphasis on new developments in relation to the detection of antibiotic resistance proteins. Within the LUMC, we are currently setting-up a new platform, based on capillary electrophoresis coupled to high resolution mass spectrometry, for in-depth proteomic analysis of bacterial colonies, enabling the identification of antibiotic resistant proteins in minute amounts of samples. As a proof of principle, we tested our method to identify bèta-lactamase peptides in an ampicillin resistant *E. coli* lab strain. At present, we are studying a variety of different clinical bèta-lactam resistant *E. coli* isolates, including extended spectrum bèta-lactamase (ESBL), in order to discriminate between the different classes of bèta-lactamases (e.g. AmpC, OXA, CTX-M).

We believe that our approach is a valuable extension of current microbiological routine methods and we anticipate that in the future it will also be applicable to identify other phenotypes, including markers for aminoglycoside resistance (phosphotransferases, adenyltransferases, acetyltransferases).

O115

Characterization of antibiotic resistance genes from a metagenomic library from a human gut microbiota enrichment culture

T.D.J. Bello Gonzalez[1], E. Bülow[2], M.W.J. van Passel[1], W. van Schaik[2], H. Smidt[1]

[1]*Wageningen University, Dept; of Microbiology, Wageningen,* [2]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht*

Introduction: The human gut microbiota comprises 10^{13} - 10^{14} bacterial cells, and plays an important role in maintaining intestinal homeostasis. When the microbiota is perturbed e.g. during the administration of antibiotics, the number of micro-organisms is reduced, and the resistance to colonization by potential pathogens is decreased, which may lead to several effects. One is overgrowth by naturally resistant micro-organisms, and a second is the establishment of new resistant pathogenic bacteria. The aim of this study was determine the presence of the antibiotic gene reservoir in the fecal microbiota of a patient who received antibiotic prophylaxis (selective oropharyngeal tract decontamination; SOD) during hospitalization in the intensive care unit (ICU).

Methods: A fosmid library (2.4 Gbp in size, insert size 40 Kb) using the pCC1FOS™ vector was constructed from anaerobic liquid culture (PYG medium) of a fecal sample collected from a patient who received topical application of polymyxin, tobramycin and amphotericin B. The composition of the fecal microbiota of this patient was determined using the human intestinal tract chip (HIT-Chip). The fosmid library was screened in *Escherichia coli* (EPI300) for antibiotic resistant clones to identify the antibiotic resistance determinants.

Results: Gut microbiota compositional analysis using the HIT-Chip revealed the presence of *Bacteroidetes* (53%), *Firmicutes* (40%, mostly *Clostridium* clusters XIVa and IV) and *Actinobacteria* (6%) as dominant phyla. The screening of the fosmid library revealed the presence of multiple genes conferring resistance to ampicillin, tobramycin, erythromycin or tetracycline. No clones resistant to imipenem were found.

Conclusion: We showed the presence of a broad range of antibiotic resistance genes in anaerobic bacteria in a patient receiving prophylactic antibiotic treatment during ICU stay. The presence of antibiotic resistance genes in the gut microbiota may enhance the possibility of resistance gene transfer from gut commensals to opportunistic pathogens.

O116

Evaluation of the efficacy of bacteriophages-derived lytic enzymes (lysins) to reduce colonization of *Streptococcus suis* in pigs

C.N.T. Dekker[1], A. Bouma[1], A.J.J.M. Daemen[1], J.C. Vernooij[1], L.A.M.G. van Leengoed[1], D.B. Gilmer[2], J.E. Schmitz[2], V.A. Fischetti[2], J.A. Stegeman[1], J.A. Wagenaar[1]

[1]*Faculty of Veterinary Medicine, Utrecht University, Farm Animal Health, Utrecht,* [2]*The Rockefeller University, Laboratory of Bacterial Pathogenesis, New York, USA*

Introduction: *Streptococcus suis (S. suis)* is an important pathogen in pigs, and may cause meningitis, arthritis, sepsis, and mortality in mainly young animals, leading to serious economic losses in pig production and compromised animal welfare. The disease is also zoonotic, and in infected humans lesions similar to those in pigs may occur. To control *S. suis* related problems large amounts of antimicrobials are used in pig production, resulting in increased antimicrobial resistance in pathogens and commensals which may also pose a human health risk. Alternative strategies are needed. One approach could be the therapeutic use of bacteriophage derived lytic enzymes (lysins). One of the advantages of lysins over most conventional antimicrobials is their high specificity; mostly genus- or species-specific. Lytic activity of lysins has been shown *in vitro* against several bacterial species, among which *S. suis,* and also in some studies in mice. In these *in vivo* studies a single dose of lysin applied on the mucosa reduced the *streptococci* (*S. pyogenes*, group B *streptococci* or *pneumococci*) by several logs. However, for none of the known lysins their *in vivo* efficacy has been evaluated in the natural host of the pathogen. Recently, lysin encoding genes were identified in enomic DNA of *S. suis*. These genes were cloned and expressed to produce the lysins fjPlySs1 and PlySs2. In this study the effect of nasal and oral application of these two lysins on *S. suis* serotype 9 colonization and transmission, and on clinical signs was investigated in the natural host of *S. suis*, i.e. pigs.

Methods: Two experiments with a similar design, which only differed in lysin doses, were performed. Each experiment consisted of one lysin and one placebo treated group. In each group 5 pigs were inoculated intranasally with $1x10^9$ colony forming units of *S. suis*, and 6 contact pigs were subsequently added. Pigs were monitored for two weeks, in which treatment was given in two periods of 2-4 days. Per treatment session a pig received a combination of fjPlySs1 and PlySs2, in low doses (0.8 and 0.4 mg) or high doses (1.1 and 3.5 mg) in the two experiments respectively. Saliva and nose swab samples, and at necropsy tonsillar tissue samples were collected and tested for *S. suis* by quantitative bacteriological culture.

Results: On 1-2 days a significant reduction of numbers of *S. suis* in saliva (1.27-1.81 ^{10}LogCFU) or nose samples (1.67 ^{10}LogCFU) was observed in lysin treated pigs. All contact pigs were colonized within 5 days. No differences were observed in the estimated transmission rates between the lysin treated and control groups ($P_{low\text{-}dose}$ = 0.530; $P_{high\text{-}dose}$ = 0.487). Clinical signs and mortality were comparable in treated and control animals.

Conclusion: Although phage lysins fjPlySs1 and PlySs2 show a clear lytic activity against *S. suis in vitro*, they appeared not to be effective in pigs with the current formulation. Application did not reduce transmission of *S. suis* between animals. There were no indications of protection against clinical signs or mortality. Reduction of mucosal colonization was only observed on some days of lysins administration.

O117

Transcriptional termination regulates the expression of the multidrug-ABC-transporter BmrC/BmrD

E. Reilman, R.A.T. Mars, E.L. Denham, J.M. van Dijl

UMC Groningen, Dept. of Medical Microbiology, Groningen

Since the first clinical use of antibiotics, bacteria have evolved several effective approaches to neutralize their activity. An efficient mechanism to achieve multi-drug resistance (MDR) is the (over)-expression of efflux pumps, such as the ATP-binding cassette (ABC)-transporters. Recently, Torres et al. characterized a new MDR-ABC-transporter in *Bacillis subtilis*, the heterodimer bmrC/bmrD. Their studies showed that expression of bmrC and bmrD was induced upon exposure of the cells to several translational inhibitors.[1] In this present study we addressed the regulatory mechanism controlling the expression of this MDR-ABC-transporter system.

To monitor the expression of the MDR-ABC-transporter system a promoter-GFP fusion was constructed. The 600-bp region upstream of bmrC was cloned into pBaSysBioII, an integrative plasmid for *Bacillus subtilis* 168.[2] By using the resulting P_{bmrC}GFP fusion strain and Live Cell Array technology, we analyzed the transcriptional activation of bmrC and bmrD in real-time and confirmed that ribosome-targeted antibiotics, such as lincomycin, indeed induce transcription of the transporter. However, the real-time approach demonstrated that the antibiotic-induced expression of the ABC-transporter genes is temporally controlled, only occurring during the late-exponential and stationary growth stages. The two ABC-transporter genes are co-transcribed with yheJ, a small, uncharacterized, open reading frame located upstream of bmrC. Transcription of the yheJ/bmrC/bmrD operon is most likely regulated from a single promotor upstream of yheJ, and therefore we constructed a PyheJGFP fusion strain. The yheJ-promoter was activated during late-exponential and stationary growth, but it proved unresponsive to the antibiotics that induced the transcription of bmrC/bmrD. This finding led us to speculate whether features within yheJ could control the expression of bmrC/bmrD. Interestingly, within the coding region of yheJ, we identified three alternative stem-loop structures, which we predicted to act as intrinsic terminator, anti-terminator and anti-anti-terminator. To test our hypothesis that these terminator structures control the transcription of bmrC/bmrD, we constructed several transcriptional GFP-fusion strains. Different truncated versions of yheJ were fused to GFP and cloned in front of a xylose-inducible promoter. Using the transcriptional GFP-fusions we have demonstrated that these terminator structures play a crucial role in the control of antibiotic-induced transcription of the downstream bmrC and bmrD genes. In addition, we showed that the translation of yheJ is essential for an effective transcriptional control.

Using both promoter- and transcriptional-GFP fusions we confirmed that ribosome-targeted antibiotics indeed induce transcription of the MDR-ABC transporter genes bmrC and bmrD. However, in contrast to the earlier findings, we demonstrated that expression is restricted to transition-and stationary-growth phases, which is tightly controlled by the promoter of the yheJ/bmrC/bmrD-operon. Antibiotic-induced transcription of bmrC and bmrD is regulated via a transcriptional termination mechanism within yheJ. Since translation is essential for the antibiotic mediated read-through, we propose that YheJ acts as a leader-peptide. We postulate that the antibiotic-induced stalling of the ribosomes during translation of the leader peptide promotes the transcription of bmrC/bmrD.

References

1. Torres, et al. Biochim Biophys Acta. 2009;1788:615-622.
2. Botella, et al. Microbiology. 2010;156:1600-1608.

O118

A novel phenotypic detection strategy for class A, B and OXA-48 carbapenemases in *Enterobacteriaceae* using temocillin

K. van Dijk[1,2], J. Scharringa[2], G. Voets[2], W.S. Voskuil[2], A.C. Fluit[2], W.C. Rottier[2], M.A. Leverstein-van Hall[3], J.W.T. Cohen Stuart[2]

[1]*AMC, Dept. of Medical Microbiology, Amsterdam,* [2]*UMC Utrecht, Dept. of Medical Microbiology, Utrecht,* [3]*Bronovo Hospital, Medical Microbiology, The Hague*

Objectives: In *Enterobacteriaceae*, class A (KPC) and B (MBL) carbapenemases can be identified with inhibition tests using boronic acid derivatives (BA) and dipicolinic acid (DPA)/EDTA, respectively. However, for class D (OXA) carbapenemases, no specific inhibitor is available, making phenotypic differentiation between OXA-48 and ESBL/AmpC production with porin changes difficult. Since OXA-48 was shown to confer high-level temocillin resistance, this study aimed to evaluate a strategy for

phenotypic detection and identification of class A, B and OXA carbapenemases using temocillin disc diffusion besides the BA and DPA inhibition tests.

Methods: The test collection included 128 well characterized non-repeat *Enterobacteriaceae* isolates suspected for carbapenemase production, i.e. with meropenem MIC ≥ 0.5 mg/L (83 *K. pneumoniae*, 17 *E. coli*, 23 *Enterobacter* spp., 3 *P. mirabilis*, 2 *S. marcescens*).The isolates produced KPC (n = 36), MBL (n = 31), KPC plus MBL (n = 4), OXA-48 (n = 25), OXA-162 (n = 2), ESBL (n = 19), AmpC (n = 10) or ESBL plus AmpC (n = 1). PCR and sequencing of bèta-lactamase genes was used as reference test. Phenotypic carbapenemase detection was performed with discs (Rosco) containing meropenem (10 ug), temocillin (30 ug), meropenem + BA, meropenem + DPA, meropenem + BA + DPA, and meropenem + cloxacillin (CL). The interpretation of carbapenemase inhibition tests consisted of two steps. First, to identify KPC and MBL producers, inhibition tests with BA and/or DPA and CL were evaluated. Second, when no synergy between meropenem and BA, DPA or both was observed, absence of an inhibition zone (≤ 10 mm) around the temocillin disc was used to identify OXA carbapenemases.

Results: For identification of class A, B and OXA carbapenemases the sensitivity was 97%, 90.3% and 100%, respectively and specificity 100%. Due to swarming, interpretation of two *P. mirabilis* isolates was false negative. Sensitivity for class B detection in non *Proteus* spp. was 97%. The sensitivity for all carbapenemase classes combined was 96% (98% in non *Proteus* spp.). None of the 27 OXA producers showed an inhibition zone around the temocillin disc. ESBL and/or AmpC producers had temocillin zone diameters between 13 to 29 mm.

Conclusion: A 30 ug temocillin disc with a zone breakpoint of ≤ 10 mm added to carbapenemase inhibition tests with BA and DPA, enables sensitive (96%) and specific (100%) detection and identification of KPC, MBL and OXA-48, the most prevalent carbapenemases in *Enterobacteriaceae*.

O119

Emerging mupirocin resistance in staphylococci following the implementation of a new *S. aureus* decolonization strategy

D.J. Hetem, H.C. Vogely, A. Troelstra, J.G. Kusters, M.J.M. Bonten

UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: The eradication of *Staphylococcus aureus* in pre-operative patients, using nasal mupirocin and chlorhexidine bodywashings, reduces the number of post-operative *S. aureus* wound infections. However, the rapid identification of *S. aureus* carriers is costly and logistically challenging. In this study, we evaluate the effects on resistance in staphylococci, of a new decolonization strategy in which all patients are treated with topical mupirocin and chlorhexidine body washings, irrespective of *S. aureus* carrier status.

Methods: Patients admitted to three surgical wards, with an expected stay of more than three days, were treated with nasal mupirocin ointment and chlorhexidine soap for five days, irrespective of *S. aureus* carrier status. Nasal swabs were obtained on: (1) admission and before the start of the decolonization treatment; (2) four days after surgery and one day after completing the decolonization treatment. Nasal swabs were inoculated on selective agars containing 8 g/ml mupirocin. All growing staphylococci were identified by MALDI-TOF and resistance for mupirocin was confirmed by E-test. Staphylococci with a minimum inhibitory concentration (MIC) > 256 were considered high-level resistant.

Results: During the five month study period 535 patients were screened, of whom in 334 (62%) nasal swabs were performed both on admission and after decolonization, four days post surgery. Overall, mupirocin resistant staphylococci were detected in 20% of the patients on admission, increasing to 42% after decolonization (p = 0.000). (Table 1) Of the 273 patients without colonization of mupirocin resistant staphylococci at admission, 105 (39%) were colonized with mupirocin resistant staphylococci after decolonization, which leads to a colonization rate of 7.7 per 100 patient days at risk. Overall, 271 mupirocin resistant staphylococci were identified of which 259 were high-level resistant. Save for one *S. aureus* isolate, all were coagulase-negative staphylococci (CoNS), mostly *S. epidermidis* (251/270, 93%).

Conclusion: Resistance against mupirocin was widespread in coagulase-negative staphylococci but rare in *S. aureus* and mupirocin resistance rates increased after patients received the decolonization regiment.

O120

Prevalence of extended spectrum bèta-lactamase-producing *Escherichia coli* in people living and/or working on Dutch broiler farms

P.M.C. Huijbers[1], E.A.M. Graat[2], A.H.A.M. van Hoek[1], M.G. van Santen[1], P. Hengeveld[1], A. Haenen[1], L.M. Schouls[1], A.W. van de Giessen[1], E. van Duijkeren[1]

[1]*National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control (CIb), Bilthoven,* [2]*Wageningen University, Quantitative Veterinary Epidemiology, Wageningen*

Introduction: ESBL-prevalence in patients (4.9%) and individuals in the general Dutch population (5.1%) were much lower than the reported 33% among broiler farmers. This suggests that contact with broilers, or the broiler farm environment, might be a risk factor for ESBL-carriage in humans. The main objectives of our studies were to estimate the prevalence of ESBL-carriage in people living and/or working on conventional and organic Dutch broiler farms, and to identify and quantify risk factors for ESBL-carriage, specifically the effect of close contact with broilers.

Methods: Two studies were performed: (1) on 50 randomly selected conventional broiler farms out of 670 registered farms, and (2) on 8 organic out of 9 registered farms. Cloaca swabs were taken from 20 broilers between 21-40 days of age (T1), and on organic farms also at 10 weeks of age (T2). Individuals living and/or working on conventional (n = 141) and organic (n = 27) broiler farms were asked to fill in a questionnaire about factors such as contact with broilers, activities in the stable, foreign travel, and medical history. Furthermore, individuals took rectal swabs at T1, and on organic farms also at T2. Human and broiler isolates were selected after growth in selective LB broth and on McConkey agar supplemented with 1g/ml cefotaxime. Bèta-lactam resistance was examined by disc-diffusion tests and bacterial species were determined. ESBL-genes were characterised by PCR and sequence analysis. Individuals and farms were classified as ESBL-positive when at least one isolate was identified genotypically as an ESBL- or CMY-2 producing *E. coli*. Risk factor analysis was done using univariable logistic regression.

Results: All conventional farms were classified ESBL-positive, with high prevalence at the sample level (T1: 96.4%). Seven organic farms were ESBL-positive at T1 with a similar prevalence at sample level of 94.3%, compared to conventional farms at T1 (p = 0.41). At T2 all farms were positive and sample prevalence was 80.0%. On conventional farms, ESBL-prevalence was 23.4% (11/47; 95% CI 12.3-38.0%), 12.8% (11/86; 95% CI 6.6-217%), and 37.5% (3/8; 95% CI 8.5-75.5%) among farmers, family members, and employees, respectively (p = 0.12). Analysis showed that close contact with broilers, and activities in the stable were not significantly associated with ESBL-carriage. Of 27 individuals sampled on organic farms, 1 was ESBL-positive (farmer) at T1, and 3 were ESBL-positive at T2 (2 farmers and 1 family member). It was not possible to draw statistically valid conclusions on risk factors for individuals on organic farms due to the low sample size and low number of ESBL-positive individuals. Preliminary analysis of isolates shows a predominance of CTX-M-1, TEM-52, and CMY-2 genes, both in human and broiler isolates.

Conclusion: 1. ESBLs are found on all broiler farms, and ESBL-prevalence at the sample level among broilers on ESBL-positive conventional and organic farms is similar.
2. On conventional farms, there seems to be no difference in ESBL-prevalence between individuals who have daily contact with broilers (i.e. famers, employees) and family members not in close contact. For organic farms the sample size was too low to draw conclusions on this.

O121

The zebrafish embryo as a novel vertebrate model for the *in vivo* analysis of biomaterial associated infection and immune responses

O.W. Stockhammer[1], X. Zhang[1], L. de Boer[1], W.J. Veneman[2], H.P. Spaink[2], D.W. Grijpma[3], S.A.J. Zaat[1]

[1]*AMC, Dept. of Medical Microbiology, Amsterdam,* [2]*Leiden University / IBL, Molecular Cellbiology, Leiden,* [3]*UTwente / MIRA, Biomaterials Science and Technology, Enschede*

Introduction: Failure of indwelling or implanted medical devices is mainly due to adverse immune reactions (bio-incompatibility) and biomaterial-associated infection (BAI), predominantly caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. To prevent infection, biomaterials with high biocompatibility and low infection-susceptibility are required. In order to test the *in vivo* biocompatibility of biomaterials in a high throughput fashion a novel animal model is desirable. Recently the zebrafish has emerged as a versatile vertebrate animal model to study immune processes and infectious diseases. It combines genetic tractability and the ability to study host-pathogen and host-biomaterial interactions *in vivo* and real time with the possibility to perform high throughput analysis.

Methods: *Fluorescent microsphere preparation*

In order to set up our zebrafish embryo model, poly (e-caprolactone) (PCL) microspheres loaded with different fluorescent dyes (blue fluorescent dye Coumarin 102 or green fluorescent dye Coumarin 6) were respectively prepared using the oil in water (O/W) emulsion and solvent evaporation technique. The particle sizes were controlled by parameter variation and subsequent sieving steps to obtain particles with desired size distributions for injection.

Zebrafish embryo implantation and infection study

Fluorescent dye loaded PCL microspheres and/or mCherry expressing *Staphylococcus aureus* were injected into the caudal region of the embryonic tail at 3 days post fertilization using a pneumatic microinjector. Injections were performed in transgenic zebrafish embryos, expressing either GFP or mCherrry (mpo:GFP, fms:nfsB.mCherry) in neutrophils and macrophages respectively.

Results: Fluorescent dye loaded microsphere were implanted into the embryo soft tissue and were clearly detectable in the embryo by fluorescent microscopy. Implantation of PCL microspheres at 2 days post fertilization led to a strong influx of neutrophils (green) and macrophages (red) within the first hours after implantation. At 3 days post implantation microspheres were

stably integrated in the host tissue and provoked only a minor cellular immune response. Tissue injection of *S. aureus* led to a rapidly progressing infection. A strong influx of neutrophils towards the infection site was observed within the first day post infection (dpi). At 2dpi bacteria were visible as confined clusters within the tissue of the embryo suggesting an uptake by phagocytes.
Conclusion: We were able to synthesize fluorescently labeled microspheres that could efficiently be injected into zebrafish embryos and traced by fluorescence microscopy. Furthermore we demonstrated that fluorescent *S. aureus* and fluorescent dye loaded microspheres permit the time-resolved visual analysis of host-biomaterial and host-pathogen interaction *in vivo*.

This research forms part of the Project P5.03 IBIZA of the research program of the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

O122
The nature and origin of resistance to antituberculosis drugs in the Netherlands in the period 1993-2011

C.J. Ruesen[1], R.G.L. van Gageldonk-Lafeber[1], G.V. de Vries[2], C.E. Erkens[2], J.R. van Rest[1], H.K.A. Korthals Altes[1], H.N. de Neeling[1], M.K. Kamst[1], D.S. van Soolingen[1]
[1]RIVM, Epidemiology and Surveillance, Bilthoven, [2]KNCV, The Hague

Background: The elimination of tuberculosis (TB) is threatened by an apparent increase in the level of resistance in *Mycobacterium tuberculosis* worldwide. Also in the Netherlands, where the majority of TB patients are immigrants, surveillance data suggest that resistance may be increasing.
Methods: We conducted a retrospective study on 18,294 notified TB cases with *M. tuberculosis* isolates between 1993 and 2011. We investigated the trends in resistance to antituberculosis drugs in this period, in relation to the origin of the patients. We distinguished resistance in new cases (primary drug resistance; PDR), which is the result of transmission of a resistant strain, and resistance in retreatment cases (acquired drug resistance, ADR). In addition, we assessed to what extent PDR was due to transmission in the Netherlands or abroad, and how often strains acquired resistance in the Netherlands or abroad.
Results: Antituberculosis drug resistance was found in 13% of all cases, and was more frequent among non-native patients (16%) than among native patients (6%, $p < 0.001$). Resistance showed an increasing trend among natives ($p < 0.001$) and a decreasing trend among non-natives ($p = 0.02$) over the period 1993-2011. After 2005, resistance has increased in both groups ($p = 0.03$ and $p = 0.01$, respectively). Although, overall, we did not observe a significant trend in resistance for the total population, we found a significantly increasing trend when streptomycin was left out ($p < 0.001$). The increase was most marked for isoniazid resistance and multidrug-resistant TB (MDR-TB) ($p = 0.01$ and $p < 0.001$, respectively). Of all resistant cases, 92% were classified as PDR and 8% as ADR. Transmission of a resistant strain happened for > 60% outside the Netherlands or before 1993. However, in 129 of 1,445 PDR cases (9%), the resistant strain was definitely transmitted in the Netherlands, and in 45 of 122 ADR cases (37%), resistance was acquired in the Netherlands.
Conclusion: 1. Antituberculosis drug resistance has increased among natives and decreased among non-natives from 1993 to 2005, where after an increase was seen in both groups.
2. Resistance to antituberculosis drugs, excluding streptomycin, showed an increasing trend for the total population from 1993-2011. As streptomycin has not been used to treat TB in the Netherlands for decades, this trend is considered highly relevant and a threat to TB control.
3. Resistance was most frequently found among non-native patients and was largely due to transmission of resistant strains, highlighting the importance of early detection and treatment programmes and early screening of resistance among immigrant patients.
4. Resistance acquisition also occurs in the Netherlands.

O126
Quality assessment by the Dutch Accreditation Council in Dutch microbiology laboratories: lessons learned and future challenges for microbiologists and managers

G.I. Andriesse
Laboratorium voor Infectieziekten, LvI, Groningen

Since the first CCKL quality guideline was published in 1991, most microbiology labs have been accredited based on this standard. Professionals wrote the CCKL guideline with expertise covering all area's of the guidelines' scope: immunology, clinical chemistry, medical microbiology, pharmacy and pathology. In other words, the CCKL guideline is a real field practice guideline made by and for professionals with a daily practice experience. The CCKL accreditation is valid for four years. Each four years the laboratory must be visited by a team of auditors and accreditation will or will not be prolonged based on the results of the audit. Today almost 90% of all microbiology laboratories in the Netherlands have been accredited. Some of the laboratories have already received prolongation of the accreditation once or twice. During the last 12 years the process of accreditation of Dutch laboratories has evolved from quality assessment for few laboratory based on a mere practice guideline 'to ISO quality accreditation as a basic standard for medical laboratories'. Today microbiology laboratories are accredited by a high standard (ISO) and by a professional accreditation body (RvA), however, in the past twelve years lessons have been learned and some argue that the bar is raised to high.
Lessons learned and future challenges for microbiologists are many: can we provide enough microbiologists to ensure independent quality assessment?, costs are rising: how about benefits?, heads of departments are sometimes little involved in their own quality system: how come?, differences between audit teams result in differences in quality assessments: is this acceptable?, in many laboratories the PDCA cycle is insufficient: what to do?

O127
What is critical for a successful audit or peer review?

M.J.E. Wieles
Dutch Accreditation Council RvA, Strategy & Development, Utrecht

More and more organisation invite persons from outside to confirm that the organisation in complying with specific requirements. This may for example be an auditor to conduct a certification or accreditation audit or a peer doing a peer review. The success of these assessment activities is depending on four main factors.
First it is important to define the objectives of the assessment and to agree on these by all parties. With unambiguous objectives the expectation of the parties involved will be managed. Part of setting the objectives is to agree on the requirements that will be used. From the objectives the roles and responsibilities of the parties involve may be derived.
The second step to ensure that the objectives of the assessment will be realised is to select a team with the necessary competence to conduct the assessment. In selecting the person or team a combination of skills and knowledge should be considered. A very knowledgeable person will not always have the necessary skills for conducting an good assessment. Also one should consider impartiality and independence issues.
A competent team will be able to realise the objectives only if the assessment process itself is managed properly. Managing the process includes planning, preparing, conduction, reporting and closing. The proper management of an assessment should ensure that appropriate interviews, file sampling and review, observation of activities, etc. will be possible.
The forth and often forgotten factor is the follow-up of an assessment. An assessment activity is a waste of resources if the results are not given the necessary consideration. This should be by a process of improvement actions, discussing the results with stakeholders and/or using the results to improve transparency and accountability.
To ensure the success of audits, peer reviews and such activities we should recognise that for the management of these kinds of activities and of the programs in which they are embedded we need professional organisations with the proper competence and drive that will be given the necessary authority. The confidence that we may have in the results of these activities may benefit from formal recognition of these organisation based on bench marking and (international) accepted standards.
Of course the question remains what is the top of the audit/review/evaluation pyramid? When will confidence based on checking be replaced by blind faith?

O128
Globally mobile populations in Europe and spread of communicable diseases

P. Gautret
IHU Méditerranée Infection, APHM Public Hospitals of Marseille, Marseille, France

Communicable diseases are costly at both the individual and societal levels. The most common infections recorded in travellers are gastrointestinal infections, febrile systemic illness including notably malaria and dengue, respiratory infections and skin infections. In addition to individual consequences, travel-associated infectious diseases can have public-health consequences if they are introduced or re-introduced by infected travellers returning to areas with susceptible populations. The international spread of malaria, dengue, chikungunya, poliomyelitis, *Neisseria meningitidis* serogroup W135 meningococcal infections, measles, influenza or Chagas disease provides strong evidence of the role of international travel in the globalization of VPDs. The surveillance of the emergence, re-emergence or spread of travel-associated diseases is essential to adapt pretravel advice and the responses to infectious threats.

O130
Monitoring changes in the resistome of travellers

C.J.H. von Wintersdorff, J. Penders, P.H.M. Savelkoul, E.E. Stobberingh, P.F.G. Wolffs
Maastricht University Medical Center, Dept. of Medical Microbiology, Maastricht

Objectives: International travelling provides an excellent opportunity for the carriage of antibiotic resistant micro-organisms from one location to another. Previous studies observed increasing antibiotic resistance after international travel when examining selected bacterial genera or species within the gut microbiome, such as the *Enterobacteriaceae*. However, very few studies have been performed studying

changes of the global resistance of the entire microbiome: the so-called resistome. This study investigates changes in the resistome of international travellers by using relative quantitative PCR.
Methods: At a local travel clinic, stool samples from 66 consenting healthy travellers were collected before and after travelling outside of Europe. Data on antibiotic usage, the destination and duration of travel, as well as complaints during or immediately after travelling were recorded. Quantitative PCRs were performed for 16S rDNA as well as for tetM and tetQ genes, encoding tetracycline resistance, aac(6')-aph(2'), encoding aminoglycoside resistance, cfxA, encoding a bèta-lactamase as well as qnrS, encoding fluoroquinolone resistance. PCR results for the resistance genes in pre-travel samples were normalised against the 16S rDNA results and subsequently compared to the normalised data obtained for the post-travel samples.
Results: The tetM and tetQ genes were present in 100% of the stool samples, whereas cfxA, aac(6')-aph(2') and qnrS were present in 94%, 66% and 28% respectively. No statistically significant changes between stools taken before and after travel were observed for the tetM, tetQ, cfxA and aac(6')-aph(2') genes. The qnrS gene, however, was detected in only 4 out of 66 (6%) pre-travel stool samples, while it was detected in 32 out of 66 (48%) post-travel samples ($p > 0.001$, OR 14.59; 95% CI 4.76-44.73). Out of the 4 pre-travel samples positive for qnrS, one was negative in the post-travel sample, and one showed a ~300-fold decrease. In the remaining two subjects, an up to 6500-fold increase in abundance in the post-travel sample was detected. For 29 post-travel stool samples, the qnrS gene was detected while it was not present in the pre-travel sample. Future work will focus on increasing the number of included travellers as well as including more resistance genes.
Conclusion: To our knowledge, this is the first study that investigates resistance genes in the gut microbiome in travellers. Results showed that resistance genes tetM and tetQ were present in all stool samples, and that cfxA and aac(6')-aph(2') genes were present in the majority of samples. The quinolone resistance encoding gene qnrS was found to be present significantly more frequently after travelling.

O131
Travelers as part of an arbovirus sentinal surveillance system; a feasibility study using 10 years of dengue diagnostics data

N.B. Cleton[1], C. Reusken[2], M. de Jong[3], J.L. Murk[1], A. van der Eijk[1], M. Koopmans[2]
[1]Erasmus Medical Center, Dept. of Virology, Rotterdam, [2]National Institute for Public Health and Environment, Centre for Infectious Disease Control, Bilthoven, [3]Amsterdam Medical Center, Microbiology, Amsterdam

Background: In a large part of the developing world, limited surveillance is performed. In laboratory information management systems, however, data on diagnostic requests is readily available and has been stored in laboratory information management systems for years. These systems are often not designed for use beyond providing information storage capacity for individual samples, but contain information that may be amenable to trend analyses. We explored the potential use of such data for systematic monitoring of trends in arboviral disease in Dutch travelers around the world, using DENV diagnostic data as a model.
Objective: The aim of this study is to evaluate the feasibility of using the information in diagnostic databases to monitor trends in arboviral exposure in Dutch travelers, using dengue virus (DENV) as a model.
Methods: Test results, and anonymous information provided by clinicians was received for 10540 diagnostic requests for 8942 patients from the laboratory information systems of the three main arboviral diagnostic laboratories in the Netherlands from January 2000 to May 2011. The data was evaluated for completeness of a predefined minimal dataset and trends in DENV positive results by travel destination. Population travel data were obtained from a commercial registry, and dengue case notification data by country from WHO DengueNet.
Results: The completeness of the minimal datasets, travel history and clinical data were low, stressing the need for reevaluation of data logging methods. Vaccination history was rarely reported (0.4%), despite its importance for interpretation of serology. Travel destination was completed for 42% of requests and trends in IgM positive tests for this subset correlated to the WHO DENV notifications, with some discrepancies. When analyzing trends by country, the Dutch Caribbean islands and Thailand showed a high correlation between the number of diagnostic requests, the number of IgM and IgG positives, and the number of WHO reported cases. For Indonesia, the diagnostic IgM trend and number of requests correlated significantly with the WHO data but with one year delay. Discrepant results were observed for Surinam, where there was no correlation between diagnostic request trends and WHO notifications. WHO notifications preceded the trends in DENV diagnoses from the pooled dataset by one year. Most discrepancies could be explained either by underreporting to the WHO, or under-diagnosis of DENV in travelers.
Conclusion: We conclude that diagnostic requests can be used to monitor trends, but that clinicians need education on the importance of providing vaccination and travel history.

O132
Biomarkers for tuberculosis vaccines

H.A. Fletcher[1], A. Keyser[2], A. Filali-Mouhim[3], A. Hawkridge[2], E. Nemes[2], T.J. Scriba[2], H. Mahomed[2], H. McShane[1], A.V.S. Hill[1], G. Kaplan[7], G.D. Hussey[2], R.-P. Sekaly[3], W.A. Hanekom[2]
[1]Jenner Institute, Oxford University, United Kingdom, [2]SATVI, University of Cape Town, South Africa, [3]Vaccine and Gene Therapy Institute, Florida, USA

Newborn Bacille Calmette-Guérin (BCG) vaccination provides incomplete protection against tuberculosis (TB) disease in childhood. A better understanding of risk of TB disease following BCG vaccination would aid the development of new, improved vaccines.
Unsupervised hierarchical clustering analysis of BCG vaccinated infants, using whole genome gene expression (illumina expression bead-chips), suggested that two distinct mechanisms may mediate protection against TB in this infant population. In one group of cases a relative abundance of gene transcripts associated with myeloid cells and inflammation correlated with prospective risk of TB disease. In the other group of cases relatively low abundance of myeloid and inflammatory gene transcripts correlated with risk.
Our results suggest that distinct mechanisms may mediate protection against TB within the same infant population. This heterogeneity may impact on the ability to identify a single biomarker of protection from TB disease.

O133
Genomics of the virus-host interaction

A.C. Andeweg[2], L. de Waal[1], H.J. van den Ham[2], M.A. Bijl[2], F. Zaaraoui-Boutahar[2], A. van Diepen[3], P.W. Hermans[4], R.J. de Boer[5], A.D.M.E. Osterhaus[2]
[1]Viroclinics Biosciences BV, Rotterdam, [2]Erasmus MC, Dept. of Viroscience, Rotterdam, [3]Leiden University Medical Center, Dept. of Parasitology, Leiden, [4]Radboud University Medical Centre Laboratory of Pediatric Infectious Diseases, Nijmegen, [5] Utrecht University, Theoretical Biology and Bioinformatics, Utrecht

The pathogen-host interaction is complex and highly dynamic. Host responses are 'tailor-made' and critical elements of these include the induction of a proper antigen specificity, magnitude, timing, and Th phenotype. Last decades many advanced molecular tools have been developed to characterize host responses in great detail. However, the multitude of genomics tools that recently became available, offer us an unprecedented view on both sides of the pathogen-host interaction. The Virgo* consortium uses genomics tools to analyze the diversity of virus genomes as well as the molecular basis of the host response to them.
Respiratory syncytial virus infections (RSV) infections are a major cause of severe respiratory tract disease in young children, immune-compromised individuals and the elderly. Despite the clinical need, no effective and safe vaccine is yet available. RSV vaccine research has been hampered by a vaccine failure in the 60's. In a vaccine trial, infants immunized with a, formalin inactivated, alum adjuvated, experimental RSV vaccine developed enhanced disease instead of being protected from RSV infection. The correlates of both, protection and development of enhanced disease are still largely unknown.
We designed specific sets of complementary *in vitro, in vivo* as well as *ex-vivo* RSV infection- / vaccination-challenge experiments in an effort to unravel the induction and regulatory aspects of distinct Th-cell phenotype responses that have been linked to RSV induced immunopathology. In our experiments we applied mRNA – and protein profiling approaches in addition to classical tools to characterize the host responses to RSV. The integrated results of the combined experiments focusing on lung epithelial cells, Th-cells and whole lung samples provide a 'high resolution' view on RSV induced host responses and allowed us to identify biomarker sets for the evaluation of newly developed experimental RSV vaccines. The generated data contribute to a better understanding of the molecular basis of virus-host interactions and supports the rational design of improved and/or novel intervention strategies for viral infections.

**Virgo is an public-private partnership of 7 academic institutions, 3 research organizations and 10 private sector companies funded by the Netherlands Genomics Initiative (NGI) and the Dutch Ministry of Health.*

O134
Autotransporter platform for the development of multivalent *Salmonella*-based vaccines

M.H. Daleke[1], C. Ten Hagen-Jongman[1], D. Vikström[2], W.S.P. Jong[1], J. Luirink[1]
[1]Abera Bioscience / VU, Molecular Microbiology, Amsterdam, [2]Stockholm University, Center for Biomembrane Research, Departm, Stockholm, Sweden

New tuberculosis (TB) vaccines are urgently needed. Attenuated *Salmonella* strains induce potent local and systemic immune responses and show great potential as live vaccine vectors for delivery of heterologous antigens. Furthermore, similar to other gram-negative bacteria, *Salmonella* naturally produces outer membrane vesicles (OMVs), which due to their immunogenicity and intrinsic adjuvant properties are interesting as vaccines. Heterologous antigens that are secreted or expressed on the bacterial surface have been shown to elicit superior immune responses compared to antigens expressed intracellularly. The autotransporter (AT) protein secretion pathway of gram-negative bacteria combines a simple

secretion mechanism with a high transport capacity, properties which make ATs attractive as partners for transport of heterologous proteins. We recently reported on the successful engineering of the *Escherichia coli* AT hemoglobin protease (Hbp) into a platform for high-density surface display and secretion of heterologous antigens in *Salmonella (*Jong et al. Microb Cell Fact. 2012). Here, we use this platform to develop novel TB vaccine candidates in the form of live *Salmonella* strains and non-living *Salmonella* OMVs that display *Mycobacterium tuberculosis* antigens on the surface of the bacteria and vesicles, respectively.

Autotransporters consist of a secreted passenger domain and a bèta-domain that mediates translocation of the passenger across the bacterial cell envelope. We created translational fusions between the passenger domain of Hbp and the well-known *M. tuberculosis* antigens ESAT-6, Ag85B and Rv2660c (Aagaard et al. Nat Med. 2011), both individually and simultaneously. We show that these antigens are efficiently and stably displayed on the surface of an attenuated *S. enterica* serovar Typhimurium vaccine strain upon chromosomal integration. Next, the fusion constructs were expressed in a hypervesiculating *Salmonella* strain and OMVs produced by this strain were isolated by high-speed centrifugation. Analysis of the isolated OMVs showed that the three TB antigens were efficiently displayed on the surface of the OMVs.

In conclusion, we show high-density display of the sizeable TB antigens ESAT-6, Ag85B and Rv2660c separately and simultaneously on the surface of live attenuated *Salmonella*, and on the surface of *Salmonella* OMVs. Importantly, this demonstrates the potential of our AT platform for the development of multivalent vaccines. *In vitro* and *in vivo* studies are underway to evaluate the immunogenicity of our TB vaccine candidates.

O135

Non-covalently cell-bound staphylococcal proteins are candidates for active or passive immunization

F. Romero Pastrana[1], G. Buist[1], J. Neef[1], D.G.A.M. Koedijk[1], J. de Groot[1], S. Engelman[2], J.M. van Dijl[1]

[1]*University Medical Center Groningen, Dept. of Medical Microbiology, Groningen,* [2]*Universität Greifswald, Institut für Mikrobiologie, Greifswald, Germany*

Introduction: Most candidate targets that have so far been selected for studies on passive or active immunization against *Staphylococcus aureus* are covalently cell wall-bound proteins. Much less attention has been attributed to non-covalently cell wall-associated proteins. Although some of these are retained in the cell wall by specific domains, the wall-binding characteristics for most of these proteins are yet unknown. Immunoproteomic analyses have shown that quite a few of the non-covalently cell wall-bound proteins and also non-predictably wall-bound proteins are immunogenic. These proteins are thus potential targets to combat *S. aureus* infections via active or passive immunization approaches.

Methods :A state-of-the-art roadmap of the *S. aureus* secretome has been generated on basis of available genome sequences, proteomics data and bioinformatics. The secretome map includes both protein transport pathways and the extracytoplasmic proteins. Available literature data combined with computer searches using MEME and BLAST were used to predict the covalently and non-covalently cell wall-bound proteins. Using a proteomics approach several non-covalently wall-bound proteins were identified. These proteins were produced in and isolated from *Lactococcus lactis* and used for cell wall-binding studies and immunogenicity testing.

Results: Different groups of non-covalently cell wall-bound proteins could be distinguished. These include proteins with specific wall-binding domains and the 'secretable expanded repertoire adhesive molecules'. The *S. aureus* strains RN4220, SH1000 and Newman were grown and the profiles of the extracellular proteome and the wall proteomes were compared using SDS-PAGE. By extraction of the wall proteins and subsequent rebinding to purified cell wall-fragments, specific non-covalently bound wall proteins from both groups could be identified. Heterologous production of these proteins in *L. lactis* resulted in non-covalent binding to this bacterium. For most of the proteins also a strong non-covalent binding to *S. aureus* cells was observed. The group of non-covalently cell wall-bound proteins, containing so-called LysM motifs, has been shown to specifically bind peptidoglycan. In different studies the LysM-containing proteins of *S. aureus* have been shown to be involved in virulence and most of them are antigenic. Using specific antibodies directed against LysM-containing proteins of *S. aureus*, it could be demonstrated that these proteins bind in a non-covalent manner to the surface of *S. aureus* cells. Using Western blotting analyses with sera from *S. aureus* carriers or heavily colonized patients, potentially relevant immunogenic *S. aureus* surface proteins that are recognized by human antibodies were identified.

Conclusion: This study shows that the group of non-covalently cell wall-bound staphylococcal proteins are immunogenic and, thus, possible candidates for active or passive immunization.

O136

The effectiveness of bacteriophages against methicillin-resistant *Staphylococcus aureus* nasal colonization in pigs *in vitro*, *ex vivo* and *in vivo*

K.M.H.W. Verstappen[1], P. Tulinski[1], B. Duim[1], A.C. Fluit[2], J. Carney[3], J.A. Wagenaar[1]

[1]*Utrecht University, Infectious Diseases and Immunology, Utrecht,* [2]*University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht,* [3]*Novolytics Ltd., Warrington, United Kingdom*

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is widely spread among animals and humans in contact with animals, but it is also present among the general community, with different prevalence depending on the geographical area. It is the major cause of infections of skin, mucous membranes and (surgical) wounds. Bacteriophages are generally specific for subsets of strains within a species, enabling the use of specific therapies, which, in contrast to antimicrobial treatment, do not affect any other bacterial species of the patient. Phages would be an excellent candidate therapeutic agent for the elimination of colonization by MRSA. The aim of this study was to compare the efficacy of bacteriophage treatment on porcine nasal colonization with MRSA *in vitro*, *ex vivo*, and *in vivo*.

Methods: The *in vitro* effectiveness of phages Fred*710 and Felix (developed for use against human MRSA strains, gel formulation), which were provided by Novolytics Ltd. (Warrington, United Kingdom), was assessed by incubating the phages with MRSA strain V0608892/1 (MLST ST398, spa-type t011, SCCmec type V) at 10^6 CFU/mL, measuring the OD_{600} every hour. Also, this strain was grown on a porcine mucosal explant as a colonization model and phages were applied to investigate the *ex vivo* efficacy of phage treatment. MRSA was enumerated to assess effectiveness. To study the *in vivo* effect, the phages were administered for 5 days, 1 dose per day to caesarean-derived, colostrum-deprived piglets (n = 8), which had been experimentally colonized with the aforementioned MRSA strain to assess the *in vivo* effectiveness. Another 8 piglets received a placebo. MRSA was enumerated from nasal samples. Six days after the last phage administration 8 piglets received a nasal ointment with mupirocin (20 mg/g) for 5 days, 2 doses per day, to eradicate MRSA.

Results: In the *in vitro* experiment, MRSA was not able to grow in the presence of phages.

On the mucosal explants the colonization with MRSA was established with approximately 10^8 CFU/explant. However, after the application of phage solution a reduction of the MRSA colonization could not be observed.

In 16 piglets, which were colonized with MRSA at a concentration of approximately 10^5 CFU/swab sample, the concentration of MRSA in the nasal cavities was not reduced after application of the phage solution or the placebo. Phages were re-isolated from the pig's noses and were still effective against the original strain and the re-isolated MRSA from the animal from which the phages were obtained. The mupirocin ointment eradicated the MRSA from the nasal cavities and from the mucosal explants.

Conclusion: 1. The MRSA strain was not able to grow in the presence of the Fred*710 and Felix phages *in vitro*.

2. The *ex vivo* explant model is in accordance with the *in vivo* results, which suggests the potential of this model for pre-animal screening.

3. The phages did not reduce porcine nasal colonization *in vivo*, in contrast to mupirocin. It is feasible to suggest that this is due to physiological properties of the pig mucosa rather than lack of efficacy from the phages.

O137

Restriction of *Mycobacterium ulcerans* to localized geographical areas: requirement for a soil-based factor for the development of new endemic regions?

K.S. Wolfe

James Cook University, School of Public Health and Tropical Medicine, Cairns, Australia

Introduction: Buruli ulcer disease (BUD), caused by *Mycobacterium ulcerans,* that is emerging as a pathogen of global significance. Once thought to be restricted predominantly to the wet tropics, BUD and new strains of *M. ulcerans* adapted to survival in temperate climates have been found in Japan and China-indicating temperate versions of the disease may well be more widespread than previously thought. *Mycobacterium ulcerans* is highly resistant to most antibiotics, making surgical excision the primary treatment modality.

Methods: Soil and water samples were collected from the Daintree River Catchment in northern Queensland, Australia – a small focal region with 3 to 4 cases of BUD per year. All samples were examined for the presence of IS2404, a mycobacterial insertion element found in *M. ulcerans* and other mycolactone-producing mycobacteria (MPMMs), as well as characterized by molecular analysis for deletions in MURD54 and MURD152-two regions on the RD1 region that can be used to characterize ecological niche preferences in *M. marinum* and *M. ulcerans*. Lastly, the species of mycobacteria present in IS2404-positive samples and all detected strains of *M. ulcerans* were characterized by VNTR analysis.

Results: *Mycobacterium ulcerans* was detected in 4% (n = 125) and 1.9% (n = 107) of water samples collected from within the endemic region, respectively. The only samples in which *M. ulcerans* was detected that were collected outside the endemic region defined by BUD cases were linked to contaminated soil from a plant originating from within this region. Soil samples obtained from the transplanted soil remained IS2404-positive and contained *M. ulcerans* over the course of the three year study and successive samples. Transplanted contaminated soil was able to support the persistence of *M. ulcerans* within a localized radius in a non-endemic area, however,

the pathogen was never detected in any other adjacent areas nor were any cases of BUD reported from the surrounding area. Furthermore, *M. ulcerans*-containing soil samples were found consistently on the southern bank of the Daintree River within the endemic region (the river defines the northern boundary), but never from samples originating from the northern bank.

Conclusion: Taken together these findings define a BUD-endemic region from *M. ulcerans*-contaminated soil and water samples and suggest the presence of an unknown permissive factor in the soil for the persistence and development of a BUD-endemic region. This represents the first time that persistence of *M. ulcerans* in endemic areas and the development of new affected regions has been linked to factor(s) in soil composition. Identification of these factors will facilitate identification of potentially at-risk regions and may also lead to a crucial understanding of growth requirements for this organism required develop methods to discourage environmental persistence and reduce exposure.

O139

A metatranscriptome analysis of antibiotic resistance genes in a microbial community under natural conditions

D. Versluis[1], M.M. Leimena[1], J. Ramiro Garcia[1], F. Hugenholtz[1], J. Zhang[1], M. Kleerebezem[1], H. Smidt[1], M. van Passel[1], W. van Schaik[2]

[1]Wageningen University, Laboratory of Microbiology, Wageningen, [2]University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: Metagenomic studies can reveal the presence of resistance determinants in samples that consist of a community of microbes. This approach, however, cannot be used to identify active functional resistance-encoding genes under natural conditions. Metatranscriptomics, in which the collective transcriptomes are sequenced, can provide for such information. Until now, the expression of antibiotic resistance genes in natural microbial communities has not yet been studied in such a comprehensive manner. This is mainly the result of the vast sequencing effort that is required to obtain metatranscriptome datasets which have sufficient sequencing depth. Here, the aim is to investigate which antibiotic resistance genes are expressed in different microbial communities using metatranscriptome data, and furthermore, to study how such expression would relate to the circumstances in that particular biological niche.

Methods: In this study, available metatranscriptomic datasets (> 500,000 reads) were collected and screened by BLAST for reads aligning to genes that are members of different classes of antibiotic resistance genes, as defined by the antibiotic resistance database (http://ardb.cbcb.umd.edu/). Metatranscriptome data included those generated from small intestinal microbiota of four humans (in-house data), coastal water and temperate forest soil, which in total encompassed 56 Gbases of sequence data. Reads were aligned at strict cut-off settings (alignment length > 40 bp, percentage nucleotide identity > 90 %, e-value < 1.0E-07). The sampled communities were not exposed to antibiotics during at least 4 months before sampling.

Results: In the human gut metatranscriptome, transcripts were detected for genes that confer resistance to -lactams, tetracyclin, erythromycin, kasugamycin, polymyxin, bacitracin and chloramphenicol. Moreover, there was evidence for the transcription of various multidrug-efflux pumps. There was a marked inter-individual difference in terms of which genes were expressed. InUSA coastal water, expression of class A bèta-lactamase encoding genes was observed. In forest soil, expression was observed of genes conferring resistance to -lactams, aminoglycosides and chloramphenicol.

Conclusion: It was shown that various antibiotic resistance genes are expressed in gut microbial communities under natural conditions. Such constitutive expression can be a remnant of prior anthropogenic antibiotic pressure. However, it could also be that resistance genes have other roles in sea water, forest soil and possibly also in gut microbiota, including roles in processes such as in (the disruption of) signalling or the modulation of gene expression. Furthermore, it could indicate that there is competition and that the antibiotics themselves as well are being produced in these biological niches. These questions are difficult to address, especially because there are no sea and gut samples available from the pre-antibiotic era. Therefore, the suggestion is to investigate the presence and abundance of members from the *Streptomyces* genus in the microbial communities, and also to investigate the presence antibiotic synthetic gene clusters.

O140

Discovery of a novel natural transformation mechanism in *Streptococcus suis*

E. Zaccaria, P. van Baarlen, J.M. Wells

Wageningen University, Animal science, Wageningen

Streptococcus suis (*S. suis*) is one of the major porcine pathogens worldwide and is considered an important zoonotic pathogen due to the high mortality observed in recent human outbreaks in China. Once bacteria enter the bloodstream they disseminate rapidly to the organs and cause septicaemia, endocarditis and meningitis. The identification of highly virulent and drug resistant serotype 2 strains in humans and pigs makes the need for cross-protective vaccines all the more urgent. Compared to other streptococci relatively little is known about the factors contributing to *S. suis* virulence in pigs and humans, this is also due to the few molecular genetic tools available to study *S. suis* virulence. We observed that *S. suis* genome sequences show the presence of a gene homologous to the *S. pneumoniae* and *S. mutans* comX gene which mediates the expression of late competence genes that enable uptake of foreign DNA. This competence mechanism is well conserved among several bacterial species and has played a major role in the evolution of bacterial genomes.

We identified in virulent *S. suis* serotype 2 genomes the set of genes involved in competence including the alternative sigma factor comX; this gene is conserved in all naturally competent streptococci and we decided to unravel its regulatory circuit. We identified a homologue of comR, the peptide signal-dependent regulator of comX in *S. mutans* and *S. thermophilus*, in the *S. suis* genome sequences. Adjacent to comR we found a small open reading frame (ORF) not annotated in the genome database. We synthesised a series of truncated peptides encoded by this unknown ORF and one of these synthetic peptides, designated as comX-inducing peptide (XIP), induced competence for genetic transformation by more than 1 million fold. In order to understand better the mechanism of competence development we tested the XIP at different growth condition and optical density, with a number of DNA and peptide concentration. Also the kinetics of the induction of competence was studied, incubating the bacterial culture with the XIP and the DNA at different time points. We hypothesize that XIP is a pheromone regulator of ComR which controls expression of ComX, the master regulator of competence for genetic transformation. In order to confirm the dependency of competence induction with those genes a comR knock-out mutant and a comX knock out mutant were made created. By optimising the conditions for XIP-induced genetic competence we have opened up new avenues for genetic studies in this important zoonotic pathogen.

O141

Drugs acting on respiratory ATP synthesis: drug synergy and potential extension of antibacterial spectrum

D. Bald

VU University Amsterdam, Molecular Cell Biology, Amsterdam

Introduction: Energy metabolism has emerged as a new, attractive target pathway for development of antibacterials, in particular against persistent bacterial pathogens.[1,2] The diarylquinoline bedaquiline (BDQ, TMC207), an ATP synthase inhibitor, is a promising candidate for shortening and simplifying treatment of (multi drug resistant) tuberculosis[3] and has recently been approved by the FDA. The combination of BDQ and the front-line drug pyrazinamide, with both drugs active on ATP synthesis[4], performed exceptionally well in mouse models.[5]

Objectives: We investigate the *in vitro* potency of the drug combination BDQ/PZA and we characterize new diarylquinoline compounds active on gram-positive pathogens.

Methods: Kill kinetics were determined for BDQ and pyrazinoic acid (POA), the active entity of the pro-drug PZA, on static cultures of *M. bovis* BCG as slow-growing mycobacterial model strain. Respresentative diarylquinoine compounds with activity on *S. aureus* and *S. pneumoniae* were subjected to binding studies with components of bacterial ATP synthase and to biochemical activity assays determining their impact on human and bacterial ATP synthesis.

Results: In kill kinetics the combination of BDQ and POA displayed significantly enhanced bactericidal activity as compared with the individual drugs. This drug combination also depleted cellular ATP pools faster, providing an explanation for the observed synergy. First experiments with the efflux pump inhibitor verapamil indicate a role of efflux pumps in the synergy between BDQ and POA.[6]

Selected diarylquinoline compounds with activity on gram-positives in planktonic state and in biofilms specifically bound to subunit c of ATP synthase thereby disturbing ATP homeostasis.[7] The compounds showed moderate selectivity in terms ATP synthesis inhibition with lowest affinity for human mitochondria, intermediate affinity for *E. coli* as gram-negative model and the highest for *S. aureus*.

Conclusion: The combination BDQ and POA is strongly bactericidal and synergistic, this drug combination seems important as backbone for future TB regimens aiming at shortening TB chemotherapy. The synergy most likely is mediated by the concerted depletion of the cellular ATP pools. Usage of respiratory ATP synthesis as target pathway appears not to be restricted to mycobacteria, but may be extended to a broader spectrum of gram-positive pathogens.

References

1. Bald, et al. FEMS Microbiol. Lett. 2010;308:1-7.
2. Bald, et al. Drug Discov. Today, in press 2013.
3. Andries, et al. Science. 2005;307:223-227.
4. Lu, et al. Antimicrob. Agents Chemother. 2011;55:5354-5357.
5. Ibrahim, et al. Antimicrob. Agents Chemother. 2007;51:1011-1015.
6. Manuscript submitted
7. Balemans, et al. Antimicrob. Agents Chemother. 2012;56:4131-9.

P001

Immature virus is a co-factor in Dengue pathogenesis

J.M. Da Silva[1], I.A. Rodenhuis-Zybert[1], S. Torres Pedraza[1], D. van de Pol[1], S. Zompi[2], E. Harris[2], J.C. Wilschut[1], J.M. Smit[1]

[1]University Medical Center Groningen, Dept. of Medical Microbiology, Groningen, [2]University of California, School of Public Health, Berkeley, USA

Objectives: There are four distinct serotypes of dengue virus (DENV) and each of these serotypes may cause disease ranging from mild febrile illness to devastating manifestations including Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Disease severity of DENV infection appears to be controlled by the presence of cross-reactive DENV antibodies directed against the envelope (E) and precursor membrane (prM) by facilitating antibody-dependent enhancement of infection. We recently reported that immature DENV turns highly infectious in the presence of prM and E antibodies. These antibodies facilitate efficient binding and cell entry of immature particles into Fc-receptor expressing cells. In addition, enzymatic activity of furin present within the endosome is critical to render the internalized immature virus infectious. To better understand the biological role of immature particles in dengue pathogenesis, we next investigated the binding and enhancing properties of acute DENV sera from patients with secondary DF, DHF and DSS towards immature DENV. Also, the binding capacity of acute sera towards immature particles was evaluated.
Methods: Immature DENV-2 strain 16681 particles were produced in furin-deficient LoVo cells. Western Blot analysis was performed to analyze the prM antibody response of the three groups of patients using immature DENV. The ratio between prM and E antibodies was quantified by means of ImageQuant TL software. By means of Elisa we determined the antibody binding towards immature DENV particles. The infectious properties of immature and standard DENV-immune complexes were investigated in FcR-expressing murine macrophage cells (P388D1), in the presence and absence of furin inhibitor and analysed by plaque assay.
Results: Antibodies against prM were present in all three groups of patients, but no significant difference was observed between the groups. Also, no difference in binding capacity of human serum towards immature DENV was observed in ELISA. A similar antibody-dependent enhancement profile was observed for sera of DF, DHF, and DSS patients
Conclusion: These results suggests that immature particles and antibodies recognizing do not trigger severe disease development. The development of severe dengue is a multi-factorial process and immature particles presumably act as a co-factor in this process.

P002

Good performance of the SpectraCellRA system for typing of methicillin-resistant *Staphylococcus aureus*

R. te Witt, N. Vaessen, D. Melles, S. Lekkerkerk, E. van der Zwaan, W. Zandijk, J. Severin, M. Vos
Erasmus MC, Medical Microbiology and Infectious Diseases, Rotterdam

Background: Typing of methicillin-resistant *Staphylococcus aureus* (MRSA) remains necessary in order to assess whether transmission of MRSA occurred and to what extend infection prevention measures need to be taken. Raman spectroscopy (SpectraCellRA (SCRA), River Diagnostics, Rotterdam, the Netherlands) is a recently developed tool for bacterial typing (Willemse-Erix et al. JCM, 2009). In this study, the of the SCRA system was evaluated for typing of MRSA strains isolated from patients who were infected with or colonized by MRSA and their household members.
Methods: 1. A well-documented collection of 113 MRSA strains, collected from 54 households. The epidemiological relationship between the MRSA strains within one household was used as the 'gold standard'.
2. PFGE was used for verification of discrepant results. Furthermore, discrepant household members were analysed for possible transmission of MRSA from another known source or via contact with a person with known risk factors.
3. Reproducibility was determined by processing three reference isolates five different times. Furthermore, 26 MRSA isolates from the study were analyzed in duplicate.
Results: 1. SCRA analysis on the strain level corresponded with epidemiological data for 108 of 113 strains; a concordance of 95.6%. When results were analyzed at the household level, results of SCRA were correct for 49 out of 54 households; a concordance of 90.7%.
2. Five discrepant results were observed. PFGE showed identical isolates whereas SCRA analysis resulted in unique isolates. Analysis for (risk of) acquisition of MRSA out of the household did not result in any plausible (risk on) transmission.
3. Reproducibility of SCRA was found to be 100%.
4. Hands-on-time for 24 samples was ~3 h for SCRA and 7 h for PFGE. Turn-around-time for 24 samples was 36-48 h for SCRA and 96 h for PFGE, with a maximum of 72 samples per day for SCRA and 50 samples for PFGE. Costs of SCRA and PFGE are comparable and are approximately 50 per sample, including personnel expenses and consumables.
Conclusion: We conclude that the SpectraCellRA system is a fast, easy to use and highly reproducible typing platform for outbreak analysis that can compete with the currently used typing techniques.

P003

Prevalence and spread of multi drug resistant *E. coli* including ST131 in different patient populations in the Euregion Meuse-Rhine

C.F.M. van der Donk[1], J.H.B. van de Bovenkamp[2], H. Bamelis[3], C.C. Driessen[1], W.M. Kalka-Moll[4], K.H. Feldhoff[5], K. Magerman[6], E.E. Stobberingh[7]
[1]*Maastricht UMC, Dept. of Medical Microbiology, Maastricht,* [2]*PAMM, Laboratory of Medical Microbiology, Veldhoven,* [3]*Limburgs Gezondheids Overleg, Provincie Limburg,* [4]*Medical Care Centre Dr. Stein and colleagues, Monchengladbach, Germany,* [5]*Gesundheitsamt des Kreises Heinsberg, Heinsberg, Germany,* [6]*Jessa Hospital, Clincal biology, Hasselt, Belgium,* [7]*MUMC, Maastricht*

Objectives: We determined the prevalence of resistance and genetic background of (multi drug) resistant (MDR) *E. coli* collected from general practice (GP) patients, and nursing home (NH) residents, intensive care unit (ICU) and urology services (URO) patients in the Euregion Meuse-Rhine, which includes border regions of the Netherlands, Belgium and Germany.
The obtained data will enable us to investigate the prevalence and spread of MDR and/or extended spectrum bèta-lactamase (ESBL) producing *E. coli* strains in this border region.
Methods: The isolates were characterized with quantitative susceptibility testing, ESBL typing, multi-locus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE). Based upon related sequence types (eBURST) and clonal frame analysis (CFA) were used to cluster the MLST sequence types (STs).
Results: A total of 1651 *E. coli* isolate were collected, ranging from 488 to 639 isolates per country and from 421-597 isolates per population except for the ICU isolates (n = 180).
The prevalence of resistance was in the same order of magnitude in the three countries but varied significantly between the different populations. Overall the GP isolates showed the lowest prevalence of resistance, among the ICU isolates the highest resistance was observed for amoxicillin (61%) and co-amoxiclav (40%) and among the URO isolates for the ciprofloxacin (18%).
The overall prevalence of MDR and/or ESBL producing isolates was 11% (n = 182), varying from 8% in theNetherlands to 14% in Belgium, of which 48 were ESBL producers. The most prevalent ESBL type was CTX-M 15 (n = 34).
Among the MDR and/or ESBL producing isolates 47 STs were found. ST131 was the most prevalent ST (74 out of 182 samples, 41%), followed by ST393 (n = 10) and ST88 (n = 9). eBURST analysis of the isolates showed 7 groups and 27 singletons. The largest group with predicted founder ST10 contained 6 different STs. ST131 could not be assigned to any group. STs that were clusterd into one CC with eBURST were also closely related according to the CFA.
Isolates with ST131, ST393 and ST88 were further analyzed with PFGE. The isolates with ST393 had comparable pulsotypes (Dice = 92%) as were the results for 31 isolates of ST131 (Dice > 80%). Seven isolates were more variable with an overall Dice similarity of =66%. The ST88 isolates showed slightly more diverse pulsotypes (Dice = 68%)
Conclusion: 1. The overall prevalence of resistance was comparable for the three countries but varied significantly between the four populations.
2. Approximately 11% of the *E. coli* isolates was MDR.
3. ST131 was the most prevalent ST.
4. Results from PFGE analysis of the ST131, ST393 and ST88 isolates suggested the spread of these isolates in the entire Euregion.

P004

Molecular identification and phylogenetic analysis of *Baylisascaris columnaris* from skunks (*Mephitis mephitis*) based on mitochondrial and ribosomal markers

F.F.J. Franssen, K. Xie, H. Sprong, J.W.B. van der Giessen
RIVM, Nationaal Referentie Laboratorium voor Parasieten, Bilthoven

Background: *Baylisascaris* species are intestinal nematodes of skunks, raccoons, bears and pandas, where fertilized females produce large numbers of eggs that are shed in the environment via the feces. After oral uptake of embryonated eggs by a wide variety of mammals and birds, larvae migrate through the body of these paratenic hosts, thereby causing considerable damage.
B. procyonis of the raccoon is known to cause severe clinical symptoms in paratenic hosts, including humans, often associated with severe or even fatal neurological disorders. Skunks, as well as raccoons, are kept as pets living in close contact with humans and sometimes besides other pets like cats and dogs. But unlike *B. procyonis*, little is known about the pathogenicity of *B. columnaris* for paratenic hosts, including humans. Moreover, to our knowledge, there are no studies available on the molecular characteristics and the phylogeny of *B. columnaris*.
Methods: In total 119 *B. columnaris* worms were isolated from pet skunks, of which 46 were used for molecular characterization. PCR primers targeted at mitochondrial cytochrome oxidase 1 and 2 (CO1 and CO2), nuclear ITS1-5.8S-ITS2 and nuclear 28S rDNA partial genes were used. Obtained PCR fragments were sequenced and used for phylogenetic analysis in comparison with sequence results following PCR of thirty-one *B. procyonis* worms from raccoons and two *B. transfuga* worms from bears.
Results: Four different multi-locus genotypes were found in *B. columnaris*. Two mitochondrial genotypes, exhibiting 10 single nucleotide polymorphisms (SNPs) in comparison with *B. procyonis*, and two nuclear genotypes. One of these was a tandem repeat on the ITS 2 gene and the other an SNP on 28S rDNA, each displaying two variants that were linked two by two between genes.
Conclusion: This is the first study presenting molecular data and phylogenetic analysis of *B. columnaris*, which was isolated from pet skunks in the Netherlands. The genetic

characteristics of *B. columnaris* show close resemblance with *B. procyonis*. Several polymorphisms were found that can be used as diagnostic sites, on both mitochondrial and nuclear markers, as described in this study. This could be used as tool to differentiate *B. columnaris* from *B. procyonis* in molecular based assays and to identify *B. columnaris* by PCR, additionally to or replacing morphometrical analysis.

P005

Dynamics of the IgG antibody response against *Staphylococcus aureus* in cystic fibrosis patients

P.M. den Reijer[1], W.J.B. van Wamel[1], H.A. Verbrugh[1], K. Becker[2], G. Peters[2], B.C. Kahl[2]

[1]*Erasmus Universiteit Rotterdam, Medische Microbiologie en Infectieziekten, Rotterdam,* [2]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

Introduction: Preliminary results show a significant correlation between the density of *Staphylococcus aureus* (*S. aureus*) in sputa cultures of patients with cystic fibrosis (CF) and both clinical symptoms and lung function of these patients. We previously demonstrated that IgG antibody levels against specific antigens of *S. aureus* are significantly increased in infected patients compared to healthy nasal carriers and non-carriers of *S. aureus*. Our current aim was to determine whether IgG antibody levels against *S. aureus* antigens could help to discriminate between colonization and infection of the respiratory tract of CF patients with *S. aureus*.
Methods: IgG antibody levels against 52 staphylococcal antigens were measured in single serum samples of 53 healthy volunteers and in multiple serum samples (median 2, range 1-9) collected over a 3-year period of 190 CF patients who were persistently colonized with *S. aureus*. Antibody levels were measured using a bead-based flow cytometry technique (xMAP, Luminex corporation). At the time of serum collection, sputa or throat cultures were taken from each patient and based on (semi-)quantitative bacterial counts in these cultures all patients were assigned to either the category infection (colony forming units, CFU > $1x10^5$) or colonization (CFU < $1x10^5$).
Results: IgG levels against 44 out of 52 *S. aureus* antigens (85%) could be reproducibly detected in patient sera. IgG levels against all antigens remained stable over time in CF patients and did not change with varying bacterial counts in sputa and throat cultures. However, the average IgG levels against 11 out of 44 antigens were significantly higher ($P < 0.05$) in patients with a high bacterial density in sputa and throat cultures (106 sera measurements) compared to patients with a low bacterial density (150 sera measurements). These antigens included the surface proteins IsdA and IsdH, the toxins leukocidin D and E, and the putative iron-regulated ABC-transporter SA0688. IgG levels against these antigens were significantly increased in all CF patients compared to healthy controls as well. A further distinction between sputa and throat cultures revealed that the differences in IgG levels between CF patients with high and low bacterial densities were no longer significant for any antigen expect Fibronectin-binding protein B when only patients with semi-quantitative bacterial densities in throat cultures were analyzed.
Conclusion: CF patients with persistent airway colonization by *S. aureus* and high bacterial densities in sputa and throat cultures have on average significantly higher, stable IgG levels to a specific subset of staphylococcal antigens than patients with a low bacterial density. Our results suggest that measuring *S. aureus* specific IgG levels together with quantitative bacterial densities in sputa could help to discriminate between colonization and infection.

P006

High resolution sequence typing to resolve repeated *Chlamydia trachomatis* infections in young heterosexual Dutch populations

S.M. Bruisten[1], R.J.M. Bom[1], M.E.G. Wolfers[2], J.S.A. Fennema[1], I.V.F. van den Broek[3], A.G.C.L. Speksnijder[1], H.M. Götz[2]

[1]*GGD Amsterdam, Public Health Laboratory, Amsterdam,* [2]*Rotterdam Rijnmond GGD, Public health service, Rotterdam,* [3]*RIVM, Epidemiology & surveillance unit, Bilthoven*

Introduction: Repeated infections of *Chlamydia trachomatis* (CT) occur frequently in young adults. These may be new infections, or persistent infections due to treatment failure or unresolved infections in the current sex partner. We aimed to establish the additional value of CT typing using high resolution multi-locus sequence typing (CT-MLST) in discriminating new from persistent repeated infections.
Methods: Paired samples at baseline (T0) and after > 6 months (T1) both positive for CT were selected from 2 Dutch screening implementation studies (CSI Amsterdam and CRI Rotterdam) among young heterosexual persons. Typing of 6 MLST loci included: ompA, CT046 (hctB), CT058, CT144, CT172 and CT682 (pbpB). The uniqueness of strains was assessed by adding 256 CT-MLST types derived from another study among heterosexuals inAmsterdam. All samples were collected between 2009 and 2011.
Results: For 27 out of 34 paired cases, full sequence types were obtained. A minimum spanning tree, including also the 256 typed reference samples, showed large and small clusters and singletons. For 17 of 27 cases a multi-locus (13 cases) or single locus variant (SLV, 4 cases) was seen, indicating new *Chlamydia* infections at T1. For 5 discordant cases by MLST the *ompA* genovar was identical. The other 10 cases with concordant typing results were categorized as treatment failure (5 cases) or persistent repeated infections (n = 5). Surprisingly, these concordant cases had *Chlamydia* strains that were either unique (singleton) or were found in small clusters. The median time between T0 and T1 did not differ between the concordant and discordant cases. The number of sex partners before T0 however, was higher for the discordant group.
Conclusion: High resolution typing was superior in discriminating new infections compared to only using ompA genovar typing. However, many cases (37%) showed exactly the same *Chlamydia* strain after 6 months. Possibly even more refined typing would still be needed. Partner treatment remains essential as well as further research on treatment failure and alternative treatment.

P007

Vibrio vulnificus strains from outbreaks in Dutch eel culture since 1996: molecular genotyping, antibiotic resistance, and zoonotic impact

R. Jansen[1], O. Haenen[2], I. Roozenburg[2], M. Engelsma[2], M. Voorbergen-Laarman[2], S. Boers[1], A.V.M. Möller[3], E. van Zanten[3], A. Dijkstra[4]

[1]*Streeklab Haarlem, Molecular biology, Lelystad,* [2]*Central Veterinary Institute of Wageningen UR, National Reference Laboratory of Fish and Shellfish Diseases, Lelystad,* [3]*Laboratory for Infectious Diseases, Groningen,* [4]*Sanquin Blood Supply, Zwolle*

Vibrio vulnificus is a zoonotic bacterial pathogen of fish. In this study, from 1996-2011, *V. vulnificus* was isolated 24 times in the Netherlands, related to nine aquaculture businesses. The *V. vulnificus* isolates were related to serious disease outbreaks in indoor eel farms and one caused an infection of the eel farmer resulting in severe zoonosis (*necrotic fasciitis*).
Objectives: To study the genetic relatedness of *V. vulnificus* strains in eel farming.
Methods: Using two genotyping techniques, MLST (using HiMLST technology) and REP-PCR (Diversilab), we studied the outbreak strains.
Results: The 24 strains could be separated into 9 HiMLST types and 8 by REP-PCR. The HiMLST and REP-PCR types corresponded almost exactly to each other. Surprisingly, only one of the 9 HiMLST types was present in the online MLST database and the other eight each had one or more new allele variations. This indicates that many, yet unknown *V. vulnificus* genotypes occur in eel farms. Most farms harboured a single genotype, and most genotypes were restricted to a single farm. However, two farms harboured a single genotype simultaneously. Eel farms sometimes buy elvers from the same supplier, which might explain this. Not surprisingly, the eel farmer that suffered from *V. vulnificus* infection carried the same genotype as the eels in his farm, a demonstration of the zoonotic potential of this genotype of *V. vulnificus*. The antimicrobial resistance patterns were diverse amongst the genotypes of *V. vulnificus* and no clear correlation was found between the genotype and the antibiotic resistance profile of a strain. Most *V. vulnificus* strains were resistant to cefoxitin and multi-resistance to quinolones, properties that were probably acquired due to prolonged use of flumequine bath and other antibiotics in eel farming.
Conclusion: As a result of this study, we propose the need for risk assessment and prevention to protect fish farmers and fish processors against *V.vulnificus* infections, particularly from eels. Furthermore, the medical branch should be informed about the potential of severe zoonotic infections of *V.vulnificus* as a human health hazard for risk individuals in our geographical area.

P008

Caenorhabditis elegans as a model system to study drug-induced mitochondrial dysfunction

R.L. Smith, R. de Boer, H. van der Spek, S. Brul

University of Amsterdam, SILS/MBMFS, Amsterdam

Introduction: Mitochondrial dysfunction is a common consequence of therapeutic drug use, especially with drugs used to treat HIV-infected individuals. Most research has been done in patient- or cell culture studies, which pose limitations on the experiments that can be performed. Progress in this field is highly dependent on the development of a robust and accurate model system.
Methods: To address fundamental questions concerning drug-induced mitochondrial dysfunction, *Caenorhabditis elegans* was applied as a model organism. Mitochondrial DNA (mtDNA) copy number was measured using quantitative Real Time PCR. Oxygen consumption rates were measured using a Neofox fiber optic oxygen sensor. Reactive oxygen species (ROS) production was quantified in a self-developed novel strain using a Biotek Synergy Mx plate reader. Mitochondrial morphology in body wall muscle cells was visualized in transgenic glo-1(zu391) animals using mito::GFP expressed from the myo-3 promoter. Image analysis was performed using ImageJ freeware.
Results: We show a concentration dependent decline in mtDNA copies when cultured in the presence of various anti-retroviral drugs. Moreover, exposure to these drugs resulted in altered aerobic respiration, increased ROS production and/or a quantifiable disruption of the mitochondrial morphological network. The severity of the observed effects is drug-specific and concentration dependent. Interestingly, the observed biochemical and morphological effects are not necessarily provoked by the same compounds and some of the effects can be

alleviated by providing supplementation with anti-oxidant compounds.
Conclusion: Our observed effects in *C. elegans* closely resemble the side-effects of drugs in patients on anti-retroviral therapy. We conclude that *C. elegans* is a highly suitable model organism to study drug induced mitochondrial dysfunction.

References

- Brinkman K, et al. AIDS. 1998;12:1735-1744.
- Carr A, et al. Lancet. 2000;356:1423-1430.
- Apostolova N, et al. Trends Pharmacol Sci. 2011;32:715-725.
- Ct HC, et al. N Engl J Med. 2002;346:811-820.
- Tsang WY, et al. Biochim Biophys Acta. 2003;1638:91-105.
- Bratic I, et al. Methods. 2010;51:437-443.
- Braeckman B, et al. Mech. of ageing and development. 2002;123:105-119.
- Bess A, et al. Nucleic acids research 2012;40:7916-31.
- Yasuda K, et al. Biochem. and biophys. research comm. 2011;404:751-755.

P009

Campylobacter bacteremia: a rare and under-reported event?

R.P.L. Louwen[1], P. van Baarlen[2], D. Horst-Kreft[1], A. van Vliet[3], A. van Belkum[4], J. Severin[1], J. Hays[1], H. Endtz[1]
[1]*Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam,* [2]*Wageningen University, Host-Microbe Interactomics, Wageningen,* [3]*Institute for food research, Gut Health and Food Safety, Norwich, United Kingdom,* [4]*BioMérieux, Microbial Research, La Balme Les Grottes, France*

Bacteria belonging to the species *Campylobacter* are the most common cause of bacterial diarrhea in humans. The clinical phenotype associated with *Campylobacter* infections ranges from asymptomatic conditions to severe colitis and bacteremia. In susceptible patients, *Campylobacter* infections are associated with significant morbidity and mortality, with both host factors and bacterial factors being involved in the pathogenesis of bacteremia. In the host, age, gender and immune-compromising conditions may predispose for *Campylobacter* infections, whilst the most important bacterial determinants mentioned in the literature are cytotoxin production and flagellar motility. The role of sialylated lipo-oligosaccharide (LOS) and serum resistance in bacteremia is inconclusive at this time, and the clinical significance of *Campylobacter* bacteremia is not yet fully understood. More emphasis on the detection of *Campylobacter* species from blood cultures in susceptible patients at risk for *Campylobacter* infections will increase our understanding of the pathogenesis and the relevance of *Campylobacter* bacteremia.

P010

Toll-like receptor 1/2 heterodimers play an important role in the recognition of *Borrelia spirochetes*

M. Oosting, H. ter Hofstede, P. Sturm, G.J. Adema, B. Kullberg, J.W.M. van der Meer, M.G. Netea, L.A.B. Joosten
Radboud University Nijmegen Medical Centre / N4i, Dept. of Medicine, Nijmegen

Introduction: After infection with *Borrelia* species, the risk for developing Lyme disease varies significantly between individuals. Recognition of *Borrelia* by the immune system is mediated by pattern recognition receptors (PRRs), such as TLRs. While TLR2 is the main recognition receptor for *Borrelia* spp., little is known about the role of TLR1 and TLR6, which both can form functionally active heterodimers with TLR2. Here we investigated the recognition of *Borrelia* by both murine and human TLR1 and TLR6.
Methods: Peritoneal macrophages from TLR1- and TLR6-gene deficient mice were isolated and exposed to *Borrelia*. Human PBMCs were stimulated with *Borrelia* with or without specific TLR1 and TLR6 blocking using specific antibodies. Finally, the functional consequences of TLR polymorphisms on *Borrelia* -induced cytokine production were assessed.
Results: Splenocytes isolated from both TLR12/2 and TLR62/2 mice displayed a distorted Th1/Th2 cytokine balance after stimulation with *B. burgdorferi*, while no differences in pro-inflammatory cytokine production were observed. In contrast, blockade of TLR1 with specific neutralizing antibodies led to decreased cytokine production by human PBMCs after exposure to B. burgdorferi. Blockade of human TLR6 did not lead to suppression of cytokine production. When PBMCs from healthy individuals bearing polymorphisms in TLR1 were exposed to *B. burgdorferi*, a remarkably decreased in vitro cytokine production was observed in comparison to wild-type controls. TLR6 polymorphisms lead to a minor modified cytokine production.
Conclusion: This study indicates a dominant role for TLR1/TLR2 heterodimers in the induction of the early inflammatory response by *Borrelia* spirochetes in humans.

P011

Human rhinovirus and enterovirus (EV68, EV104, EV105 and EV117) respiratory tract infections in symptomatic and asymptomatic adults in Europe during three consecutive winter seasons (2007-2010)

K.Z. Zlateva[1], F.C. Coenjaerts[2], T.V. Verheij[2], P.L. Little[3], H.G. Goossens[4], M.I. Ieven[4], E.C. Eric[1]
[1]*LUMC, Medische Microbiologie, Leiden,* [2]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht,* [3]*University of Southampton, Primary Care and Population Sciences, Southampton,United Kingdom,* [4]*University of Antwerp, Vaccine & Infectious Disease Institute, Antwerp, Belgium*

Introduction: Human rhinoviruses (HRVs) are classified into three species: HRV-A, HRV-B and HRV-C and are the major cause of respiratory tract infection in humans. In addition human enteroviruses (HEV), which are closely related to HRVs and classified together in the same enterovirus genus, are increasingly reported as a causative agent of acute respiratory illness. The disease outcomes associated with different HRV species are poorly established. Several studies have reported high incidence and more severe illness caused by HRV-A and HRV-C viruses, however the majority of them have focused mainly on hospitalized pediatric patients during one season. The objective of this study was to investigate the molecular epidemiology of HRV infections among adults with respiratory tract illness and asymptomatic matched controls during three consecutive winter seasons.
Methods: Nasopharyngeal swabs provided by 16 primary care networks across 11 European countries, as part of the GRACE study (Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe) were included in this study. A total of 766 samples collected during the three winter seasons (2007-2010) and positive for HRV as determined by real-time RT-PCR were selected for further characterization. The HRV positive specimens were obtained from 674 adults with acute respiratory illness during the first (V1 samples = 583) and/or follow-up visit 4 weeks later (V2 samples = 111) to the general practitioner. In addition, 72 samples were from matched controls. Genotyping of picornaviruses was performed by sequence analysis of the capsid protein genes VP1 or/and VP4-VP2, or the 5'NTR.
Results: A total of 720 (94%) picornavirus infections were successfully genotyped including: 435 (60.4%) HRV-A, 108 (15%) HRV-B, 146 HRV-C (20.3%). In addition cross-reactivity of the HRV real-time assay with HEV sequences resulted in 31 (4.3%) HEV positive samples. 18 HEV infections were cause by HEV-C serotypes EV104 (10), EV105 (5), EV117 (2) and 1 infection determined only on species level. HEV-D serotype EV68 was identified in 13 subjects. Recurrent infections with a different rhinovirus species were observed in 7 patients including 3 HRVA/HRVB and 4 HEVA/HRVC reinfections. Protracted rhinovirus infection lasting for at least 4 weeks was confirmed by 99.9-100% VP1 or VP4-VP2 nt identity of HRVs obtained from corresponding V1/V2 samples in 5 patients; 2 cases infected by a HRV-A strain and 3 infected with a HRV-B strain. Statistical analysis conducted on 456 rhinovirus single infections revealed HRV-A to be significantly more frequently associated with symptomatic infection versus HRV-B and HRV-C. HEV asymptomatic infections were identified in 5 patients: 2 EV104, 1 EV105, 1 EV117 and 1 EV-68. HEV single infections were associated with a URTI in 7 cases (1 EV104, 1 EV105, 5 EV68) and a LRTI in 8 cases (2 EV104, 1 EV105, 5 EV68).
Conclusion: HRV-A was the most frequently detected rhinovirus species and is significantly more frequently associated with respiratory disease symptoms than HRV-B and HRV-C infections. Both HRV-A and HRV-B strains can cause protracted infections in healthy adults. EV104, EV105 and EV68 infections can lead to serious LRTI in immunocompetent adults. The clinical significance of EV117 remains to be established.

P012

Detection of *Clostridium difficile* with the BD-MAX™ system

I.O.M. op den Buijs, R.T.J.M. Roymans, H.T. Tjhie, J.H.B. van de Bovenkamp
PAMM, Microbiology, Veldhoven

Objectives: Rapid and sensitive laboratory diagnostic testing is highly desirable for appropriate detection and treatment of *Clostridium difficile* (Cd) infection. The BD-MAX™ (Becton Dickinson) is a fully automated molecular platform for the extraction of nucleic acids followed by real-time PCR. In this evaluation the performance of the BD-MAX™ Cdiff assay is compared to the Immunocard toxins A&B (Meridian).
Methods: The BD-MAX™ Cdiff assay was tested on 100 stool samples (stored at -80 °C for a maximum of one year) that were positive by Immunocard toxin A&B and confirmed by the cytotoxicity assay (CTA) on HEL cells. A prospective study was performed on 556 fresh stool samples. Discrepant analysis was performed by CTA and toxigenic culture (TC). The TC was performed on a ChromID™ *C.difficile* (Biomrieux) followed by subculture of suspicious colonies in Brewer media. After incubation, 100 l of the Brewer media was added onto 1 ml of HEL cells with and without Cd anti-toxin. Cytopathic effect (CPE) was read after an overnight incubation at 372 °C. Samples were regarded as positive if CPE was present in cells without Cd antitoxin and not present in cells with Cd antitoxin. True positive samples are samples positive by either CTA or TC.
Results: None of the frozen samples and 5.4% of the fresh stool samples were inhibited in the BD-MAX™ Cdiff assay. Of the inhibited samples, 70% were resolved by rerunning from the same sample buffer tube and 30% were resolved by inoculating a new sample buffer tube with stool. In the retrospective study all 100 samples were positive in the BD-MAX™ Cdiff assay. The prevalence of true positive samples in the prospective study was 8.3% (46/556). The sensitivity, specificity, positive and negative predictive values of the BD-MAX Cdiff assay were 100%, 98.6%, 86.8% and 100% and for the Immunocard toxins A&B 47.8%, 99.6%, 91.7% and 95.5%.
Conclusion: The Cdiff assay on the BD-MAX is a highly sensitive method to detect Cd from stool samples. Because of a minimum of hands-on time and the use of a walk-away

system this assay easy fits in every microbiological laboratory setting.

P013

Migration of a laboratory designed assay for the detection of *M. tuberculosis* complex to the automated BD-MAX™ system

I.O.M. op den Buijs, R.T.J.M. Roymans, A.R. Jansz, J.H.B. van de Bovenkamp
PAMM, Microbiology, Veldhoven

Objective: The increasing number of molecular tests leads to more work and the amount of hands-on time becomes an important issue. The purpose of this study is to migrate an assay for the detection of *Mycobacterium tuberculosis* complex (MTB) onto the BD-MAX™ (Becton Dickinson) and confirm the quality of the assay by testing a panel from the Quality Control for Molecular Diagnostics (QCMD).
The BD-MAX™ is a fully automated molecular platform for the extraction of nucleic acids from different kinds of samples followed by qPCR.
Methods: In the current method samples are liquefied, DNA is extracted using the NucliSENS EasyMAG (bioMrieux) and analysed by qPCR on a 7500 Fast Real-Time PCR System (Life Technologies™) using TaqMan Fast Universal PCR Mastermix (Applied Biosystems). In the assay on the BD-MAX™ samples are liquefied and transferred into the sample buffer tube. Extraction and PCR are performed using the ExK™ DNA-1 kit and DNA MMK(SPC) from BD.
After small adjustments to the PCR protocol on the BD-MAX™, the sensitivity of the assay was confirmed to be equal to the current method. To evaluate the assay on the BD-MAX™ the MTB2011 QCMD panel (Qnostics) was tested. This panel consists of five sputa samples and five protein rich buffer (PRB) samples with various concentrations of MTB. The samples are treated according to the QCMD protocol. Briefly: PRB samples are treated like cerebrospinal fluid (i.e. no pre-treatment) and sputa samples are treated with proteinase K. Subsequently the total sample volume is transferred to the buffer tubes and incubated for 15 minutes at 95 °C.
Results: Four out of five sputa samples were positive in the MTB assay, which is in concordance with the final QCMD report. Surprisingly all the PRB samples were inhibited. Negative PRB (Qnostics) was used to test the effect of proteinase K treatment and the 95 °C heating step. In fact heating the PRB causes inhibition, which could be resolved by proteinase K treatment before heating or by omitting heating. When the PRB samples were retested using MTB assay without heating, four of the samples were positive. This resulted in a 100% score in accordance with the final QCMD report.
Conclusion: This study demonstrates the ease of migrating a laboratory developed assay onto the BD-MAX™. QCMD samples in PRB should be treated with proteinase K or heating should be omitted. The assay for the detection of *M. tuberculosis* complex had a 100% score for the QCMD MTB panel of 2011.

P014

Interaction of multi-drug resistant *Enterococcus faecium* with intestinal epithelial cells

A.P.A. Hendrickx, M.J.M. Bonten, R.J.L. Willems
University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: The incidence of infections caused by multi-antibiotic resistant *Enterococcus faecium* continues to escalate in hospitals world wide. The majority of clinical and hospital outbreak associated (termed HA) isolates are enriched with genes of which the gene products may favour attachment to and colonization of host tissues. However, the interaction of *E. faecium* with intestinal epithelial cells (IECs) has not been studied. The objective was to determine and visualize the adhesion capability of HA- and non-HA-*E. faecium* isolates to IECs.
Methods: Confluent intestinal epithelial adenocarcinoma HT-29 monolayers were maintained in culture medium for 7 days. Adherence of 5 HA-*E. faecium* and 5 non-HA-*E. faecium* strains to IECs in the presence and absence of 10 mM $CaCl_2$ was assessed for 1 hour at 37 °C, CFUs were enumerated, and data plotted as percentage of adhesion. *E. faecium* growth curves were determined in TSB broth using a Bioscreen plate reader. Scanning electron microscopy (SEM) was employed to visualize the association of the *E. faecium* cells with HT-29 IECs.
Results: All 10 *E. faecium* isolates adhered to differentiated HT-29 IECs with strain-dependent variation, ranging from 6% to 62%. Addition of 10 mM $CaCl_2$ in the assay significantly increased *E. faecium* adherence to HT-29 IECs by 1.5 to 5 fold. In particular, 10 mM $CaCl_2$ especially increased adherence of the 5 HA-*E. faecium* isolates (on average 69% adhesion) to IECs when compared to the 5 non-HA-*E. faecium* strains (on average 38% adhesion, $p < 0.05$). This observed difference was not associated with variations in growth in TSB broth with or without $CaCl_2$. SEM confirmed the adhesion assays and demonstrated association of *E. faecium* with apical microvilli of the IECs.
Conclusion: (1) We showed that *E. faecium* can interact with microvilli of HT-29 IECs and (2) that addition of 10 mM $CaCl_2$ significantly enhances enterococcal adherence to these cells. The increased ability of HA-*E. faecium* to adhere to IECs may contribute to enhanced pathogenesis in hospital-related infections, which in part may explain its emergence as nosocomial pathogen.

P015

Cytomegalovirus DNA detection in dried blood spots and perilymphatic fluids from pediatric and adult cochlear implant recipients with prelingual deafness

J.J.C. de Vries[1], A. Vesseur[2], L.J. Rotteveel[1], A.M. Korver[1], L.G. Rusman[1], E. Wessels[1], A.C.M. Kroes[1], E.A. Mylanus[2], A.M. Oudesluys-Murphy[1], J.H. Frijns[1], A.C.T.M. Vossen[1]
1LUMC, Dept. of Medical Microbiology, Leiden, 2Radboud UMCN, Nijmegen

Background: Congenital cytomegalovirus (CMV) infection is the leading cause of non-genetic congenital hearing loss. The contribution of congenital CMV to prelingual deafness and the pathophysiology is largely unknown.
Objective: 1. To analyze the prevalence of congenital CMV among cochlear implant (CI) recipients with prelingual deafness.
2. To genotype CMV present in dried blood spots (DBS) and in the inner ear years after birth.
Study design: Children and adults with prelingual deafness who received a CI in 2010-2011 were included prospectively. Perilymphatic fluids were collected during CI surgery and, in the pediatric cases, DBS were retrieved for CMV DNA detection. Furthermore, a cohort of children with prelingual deafness who received a CI between 2003 and 2008 were included retrospectively. CMV detection in DBS and perilymph was followed by gB and gH genotyping.
Results: Seventysix pediatric CI recipients were included. Seventy DBS were tested for CMV DNA, resulting in a prevalence of congenital CMV of 14% (10/70). Perilymphatic fluid was available from 29 pediatric CI recipients. One perilymph fluid, of a 21-month old girl with congenital CMV, asymptomatic at birth, was CMV DNA positive. The CMV strain in the perilymph was genotypically identical to the strain present in her DBS (gB1/gH2). Perilymph samples from 21 adult CI recipients were CMV DNA negative.
Conclusion: Our study stresses the important contribution of congenital CMV among pediatric CI recipients. Furthermore, our genotyping data support the hypothesis that CMV-related hearing loss is associated with ongoing viral replication in the inner ear up to years after birth.

P016

The Dutch Q fever outbreak in perspective: genotyping as a tool for source tracing and epidemiological investigation

J.J.H.C. Tilburg[1], H.I.J. Roest[2], A.M. Horrevorts[1], M.H. Nabuurs-Franssen[1], C.H.W. Klaassen[1]
[1]Canisius Wilhelmina Hospital, Dept. of Medical Microbiology & Infectious Diseases, Nijmegen, [2]Central Veterinary Institute of Wageningen, Dept. of Bacteriology and TSE's, Lelystad

Objectives: *Coxiella burnetii*, the causative agent of Q fever in humans, is a zoonotic gammaproteobacteria with increasing interest in Europe due to the number and proportion of reported outbreaks. In the Netherlands, more than 4000 human cases and 25 notified deaths related to Q fever were reported between 2007 and 2012. Previous genotypic studies displayed a correlation between human Q fever cases and goats/sheep. The aim of this study was to display a correlation between the Dutch Q fever outbreak genotype and other European genotypes and to build up a Q fever genotyping database in combination with a rapid typing system.
Methods: Different multiple locus variable number tandem repeat analysis (MLVA) panels and multispacer sequence typing (MST) were performed to characterize the Dutch genotypes and genotypes from human and animal clinical samples (antelope, caprine, cattle and ovine), cows' milk and ticks obtained from Canada, Dubai, France, Germany, Italy, Portugal, Qatar, Russia, Saudi Arabia, Slovak Republic, Spain, Switzerland, United Kingdom and USA.
Results: Genotypic characterization displayed a correlation between the predominant Dutch genotype and genotypes from France and Germany. Recently, this predominant Dutch genotype has also been identified in one placenta from cattle, implicating that cattle may also be a reservoir of the endemic Dutch genotype. One different genotype, obtained from a Dutch chronic Q fever patient, displayed a correlation with chronic Q fever patients from France and Portugal and to goats and sheep from Portugal and Spain. Our study determined the suitability of a 6-locus MLVA panel as a consolidated rapid typing system. All generated genotyping data were combined in an 'in-house' database containing 64 different *C. burnetii* genotypes from 241 samples. This database can be used for an interoperable web-based analysis and data collection platform available for all laboratories.
Conclusion: Molecular characterization of *C. burnetii* genotypes is important to understand the local distribution/epidemiology of *C. burnetii* throughout Europe, critical to identify the major sources of infection and implement efficient farm-based control measures to reduce human exposure to the pathogen and to prevent outbreaks. Genotyping techniques should be harmonized so that results can be exchanged between different laboratories. An interoperable web-based database can assist in the identification of new emerging *C. burnetii* strains.

P017

Prevalence and resistance of commensal *Staphylococcus aureus*: a European cross-sectional study

C.D.J. den Heijer[1], E.M.E. van Bijnen[2], W.J. Paget[2], M. Pringle[3], H. Goossens[4], C.A. Bruggeman[1], F.G. Schellevis[2], E.E. Stobberingh[1]
[1]Maastricht University Medical Centre, Dept. of Medical Microbiology, Maastricht, [2]NIVEL, The Netherlands Institute for Health Services Research, General Practice, Utrecht,

[3]*University of Nottingham, Primary Care, Nottingham, United Kingdom,* [4]*Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Medical Microbiology, Antwerp, Belgium*

Introduction: Information on the prevalence of *Staphylococcus aureus* resistance has mainly been obtained from invasive strains, although the commensal flora is considered an important reservoir of resistance. Within The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance' (APRES) study, funded by European Commission 7th Framework Program, we determined the prevalence of nasal *S. aureus* carriage and antibiotic resistance, including meticillin-resistant *S. aureus* (MRSA), among healthy patients in nine European countries. The genotypic structure of the isolated MRSA strains was determined by means of spa-typing.
Methods: From November 2010 to August 2011, nasal swabs (4,000 per country was aimed at) were obtained from patients recruited by general practitioners (GPs) participating in existing nationwide GP networks. Patients were eligible when they visited their practice for a non-infectious condition and had not been exposed to antimicrobial agents or healthcare settings in the previous three months. Swabs were sent to national microbiological laboratories for isolation and identification of *S. aureus*. Antibiotic resistance testing and spa-typing was performed at one central microbiological laboratory. Putative MRSA strains were confirmed by polymerase chain reaction (PCR). To control for the known influence of age and sex on the prevalence of *S. aureus* nasal carriage and the possible clustering of *S. aureus* carriage at a GP level, we calculated the *S. aureus* prevalence for each country using a multilevel logistic regression model. The multilevel model had three levels (country, GP and patient).
Results: *S. aureus* was isolated from 6,956 out of 32,206 patients swabbed (21.6%). The adjusted *S. aureus* prevalence ranged from 12.1% (95% confidence interval: 9.7-15.1) (Hungary) to 29.4% (CI 24.6-34.8) (Sweden). Except for penicillin, the highest resistance was observed to azithromycin: range 1.6% (CI 1.0- 2.6%) (Sweden) to 16.9% (CI 14.6-19.6) (France). No resistance was observed to linezolid and vancomycin. In total, 91 MRSA strains were isolated, with the highest MRSA prevalence being found inBelgium (2.1%, CI 1.2- 3.6). 53 different spa-types were observed, with t002 (9.9%, 9/91) and t008 (7.7%, 7/91) being most prevalent. With the exception of two Dutch MRSA strains, MRSA strains with similar spa-types belonged to patients from different GP practices. Five out of the nine Dutch MRSA strains isolated, belonged to the livestock-associated spa-CC 011 (which corresponds to MRSA CC398). All MRSA strains belonging to spa-CC 011 (n = 10) were resistant to tetracycline, as against 173% (14/81) of the non-spa-CC 011 MRSA (OR 1.71, 1.22-2.40).
Conclusion: The prevalence of resistance, including MRSA, was low among healthy individuals recruited from general practices in nine European countries. MRSA strains found showed genotypic heterogeneity, both within and between countries, implicating limited spread of MRSA in the community. MRSA CC398 dominated among the Dutch MRSA strains. The prevalence of *S. aureus* nasal carriage differed between nine European countries, even after correcting for sex, age and GP.

P018

Revised national guideline on prevention of methicillin-resistant *Staphylococcus aureus* transmission in Dutch hospitals

I.J.B. Spijkerman[1], A.K. van Vliet[1], T.M. Bonten[2], T. Daha[1], R. Hendrix[3], J.A.J. Kluytmans[4,5], B.M. Roede[1], P.J. van den Broek[1], C. Vandenbroucke-Grauls[4], M. van Rijen[5], H. Verbrugh[6], M.C. Vos[6], A. Voss[7], M.W.H. Wulf[8]
[1]*Leiden University Medical Center, Dutch Working Party on Infection Prevention, Leiden,* [2]*UMC Utrecht, Utrecht,* [3]*Laboratory Medical Microbiology Twente Achterhoek, Enschede,* [4] *VUMC, Amsterdam,* [5]*Amphia Hospital, Breda,* [6]*Erasmus MC, Rotterdam,* [7]*UMC St Radboud and CWZ, Nijmegen,* [8] *Viecuri Medical Centre, Venlo*

Introduction: The Dutch Working Party on Infection Prevention develops national guidelines for healthcare facilities, primarily hospitals. Recently, the guideline 'methicillin-resistant *Staphylococcus aureus* (MRSA)' was revised in order to modify the control measures based on current knowledge.
Methods: The MRSA guideline of the Dutch Working Party on Infection Prevention defines the search-and destroy strategy for the prevention of transmission of MRSA in Dutch hospitals. We identified key topics, performed a literature search and determined whether the guideline of the Dutch Working Party on Infection prevention published in 2007 should be modified.
Results: The increasing number of MRSA-positive patients not identified by the risk categories defined in the former MRSA-guideline (MRSA of unknown origin: about 25%) is a national concern. Literature identified two new high risk groups namely patients with a MRSA-positive household member and patients working and/or living on farms with broiler chickens. The introduction of rapid diagnostic detection methods resulted in less stringent isolation measures (contact isolation in a private room) on admission of patients belonging to the MRSA risk groups. If preliminary test results take more than 24 hours patients should be placed in strict isolation.
Patients should also be placed in strict isolation when the test is positive. Screening is mandatory in case of unprotected contact with a MRSA-positive person. Hospitals experienced increasing problems with the measures advised in the outpatient clinic, especially in areas with a high prevalence of life-stock associated MRSA. Based on one study, additional measures in outpatient clinics were considered obsolete in this setting when general precautions are taken. Moreover, the expert group identified knowledge lacunae in transmission of MRSA. One urgent matter that should be addressed are employees living on farms whom are colonized with or at risk for life-stock associated MRSA. More research is necessary to identify MRSA of unknown origin in order to maintain the low prevalence of MRSA in the Netherlands.
Conclusion: The national guideline on MRSA was revised. Several risk groups were added, isolation measures were adapted if rapid diagnostic detection methods were introduced and measures were abolished in the outpatient clinic.

P019

Prevalence of *Brachyspira* species in stool-samples of patients with gastro-enteritis

L.J. Westerman[1], R.F. de Boer[2], J.H. Roelfsema[3], I.H.M. Friesema[3], L.M. Kortbeek[3], J.A. Wagenaar[4], M.J.M. Bonten[1], J.G. Kusters[1]
[1]*University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*Laboratory for Infectious Diseases, Dept. of Research & Development, Groningen,* [3]*National Institute for Public Health, Centre for Infectious Disease Control, Bilthoven,* [4]*Dept. of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht*

Objectives: *Brachyspira* can colonize the colon of both humans and animals. Several *Brachyspira* species are known, some are recognized pathogens while others are harmless commensals. *Brachyspira pilosicoli* is known to cross-infect both animals and humans. It is the causative agent of porcine intestinal spirochaetosis, characterised by chronic diarrhoea and colitis. In humans, three species have been described: *B. aalborgi*, *B. hominis*, and *B. pilosicoli*. It has been suggested that *B. pilosicoli* causes chronic abdominal complaints in humans. Based on its established pathogenic role in animals and its association with inflammatory changes in humans we postulate that *B. pilosicoli* represents a human intestinal pathogen, and the other species are commensals. However, their role in gastro-enteritis is not well described. The aim of this study was to investigate the prevalence of *Brachyspira* species in samples from patients suffering from gastro-enteritis and controls.
Methods: A case/control-study has been performed in the late 1990's to estimate the prevalence of known gastro-enteral pathogens, and identify risk factors for micro-organism-specific gastro-enteritis in general practices. Here we used the faecal samples of this collection. DNA was extracted using NucliSens easyMAG (bioMrieux). A validated real-time PCR targeting the 16S rRNA gene of all Brachyspira species was used to investigate the prevalence of *Brachyspira*. 1,197 samples could be included, comprising 733 (61.2%) cases and 464 (38.8%) controls.
Results: Twenty-nine samples were positive for *Brachyspira* species: 17 cases (2.4%) and 12 controls (2.7%). There were 18 samples positive for *B. aalborgi* (11 cases and 7 controls) and 7 positive for *B. hominis* (4 cases and 3 controls), and 4 samples were positive for both *B. aalborgi* and *B. hominis* (2 cases and 2 controls). No *B. pilosicoli* was found in any of the included samples. No significant correlation was found regarding case/control, sex, age, symptoms, season, year, urbanization, duration of symptoms, or co-infections with other enteropathogens.
Conclusion: This is the first case/control study into the prevalence of the different *Brachyspira* species in gastro-enteritis. We did not find a difference between the presence of *Brachyspira* species in cases and controls. This confirms our hypothesis that these two species do not represent intestinal pathogens. We did not find any *B. pilosicoli* in this population. This is remarkable since *B. pilosicoli* represents ~25% of human intestinal spirochaetosis cases in studies on colon biopsies with chronic gastrointestinal complaints.

P020

Evaluation of a commercial real time PCR for the detection of extended spectrum bèta-lactamase (ESBL) genes (Check-MDR ESBL)

L.E. Willemsen[1], L. Hille[1], A. Vrolijk[1], A. Bergmans[2], J. Kluytmans[3]
[1]*Amphia hospital, Laboratory for Microbiology and Infectioncontrol, Breda,* [2]*Sint Elisabeth Hospital, Laboratory for Microbiology, Tilburg,* [3]*VUmc Medical Center, Laboratory for Microbiology, Amsterdam*

Rapid and accurate testing of multi drug resistant micro-organisms is of great importance for appropriate treatment and for the control of transmission within the hospital. In this study we investigated the performance of a rapid real-time PCR (rt-PCR) for the detection of extended spectrum bèta-lactamases (ESBL) genes in *Enterobacteriaceae*.The strains were derived from a collection of highly resistant micro-organisms that had been prospectively collected in 13 Dutch hospitals. A total of 489 ESBL positive and negative *Enterobacteriaceae*, of different species with a majority of *Escherichia coli*, were tested. The strains have previously been well characterized with respect to their bèta-lactamase genes using a micro-array (Check-KPC ESBL). The principle of the Check-MDR ESBL is based on specific recognition of target sequences by DNA probes, followed by ligation and rt-PCR, and generates results

within 4.5 hours. Analyses were performed according to the instructions of the manufacturer. Discordant isolates were retested in the PCR and tested with an extensive micro-array (Check-MDR CT103). Results from the Check-KPC ESBL in combination with the Check-MDR CT103 micro-array were considered as gold standard.One ESBL negative isolate (wildtype TEM) and 1 ESBL positive isolate (TEM238S+wildtype) showed inconclusive results, even after retesting. After comparing the results of PCR with Check-KPC ESBL, we found 31 discordant isolates. Of those, 28 PCR results corresponded to the results from the Check-MDR CT103 and were considered identified correctly. This leaves 3 discordant isolates. Three ESBL positive isolates could not be confirmed by rt-PCR because DNA probes for these genes are not included in the assay (TEM238S+wildtype, n = 2; SHV238A+240K, n = 1). This results in a sensitivity of 99,2% (351/354) and specificity of 100% (133/133).This study shows a high sensitivity and specificity for Check-MDR ESBL, a rapid molecular test for the detection of ESBL genes. This test could improve the speed and quality of the detection of ESBL and can thereby improve therapy and infection control.

P021

Respiratory desease and *Trichomonas vaginalis* in premature newborn twins

M.J. Bruins, H.L.M. van Straaten, G.J.H.M. Ruijs
Isala klinieken, LMMI, Zwolle

Introduction: *Trichomonas vaginalis* (Tv) is known as a cause of vulvovaginitis with vaginal discharge, and is associated with adverse pregnancy outcomes. Newborn children from mothers infected with Tv sometimes develop urinary tract or vaginal infection, but Tv is rarely found in the respiratory tract.
Methods: We describe premature newborn twins with respiratory disease who were diagnosed with Tv by a chance finding in a sputum sample.
Case report: The male and female twins, 25-3/7-week, 900 g, and 25-4/7-week, 870 g, respectively, were born to a 25-year-old, asymptomatic woman. Both infants were suffering from respiratory distress and were intubated. Empiric treatment with ampicillin and ceftazidim was started. On the sixth day of life *Ureaplasma urealyticum* was detected in the boy's tracheal aspirate and both children were started on erythromycin. The respiratory complaints did not resolve however. On the tenth day of life a sputum sample from the boy was tested for *Chlamydia trachomatis* (Ct) using a multiplex real-time PCR for the detection of Ct, *Neisseria gonorrhoeae* and Tv. The reaction was positive for Tv. Infection with Tv was confirmed by positive PCR for urine and vaginal samples from the boy and the girl, respectively. Additionally, a nasopharyngeal aspirate from the boy was microscopically positive for Tv. Both children were treated with metronidazol. A vaginal swab of the mother also tested positive for Tv. After treatment Tv was no longer detectable in the children. When after several days signs of pneumonia increased however, cytomegalovirus was found in urine and sputum samples and in the mother's breast milk. This was successfully treated with ganciclovir.
Conclusion: Proof of respiratory infection in neonates caused by transmission of Tv during birth is scarce. In our two cases pathogenesis was further complicated because several infectious agents played a role. Tv should be taken into account however as a cause of respiratory disease in neonates and modern molecular detection techniques can help diagnose this.

P022

Surveillance of pertussis in the Netherlands: monitoring the impact of recent changes in the vaccination program

A. Zeddeman, N.A.T. van der Maas, M. van Gent, S.C. de Greeff, F.R. Mooi, H.E. de Melker
RIVM, LIS, Bilthoven

Introduction: Because of a pertussis-upsurge since 1996, an acellular booster was added to the NIP in late 2001. Furthermore, an acellular vaccine replaced the whole cell vaccine at infancy in 2005. We aimed to measure the impact of these interventions on the Dutch pertussis-epidemiology.
Methods: Disease-, immuno- and pathogen-surveillance were used for monitoring. The screening'-method was used to calculate age-specific vaccine-effectiveness.
Results: Overall mean incidence of notifications per 100,000 increased from 32 (1996-2004) to 37 (2005-2010). Mean incidence in 0-year-olds decreased from 123 (1996-2004) to 88 (2005-2010). Likewise, mean incidence in 1-4-year-olds decreased from 123 to 52. In 5-9-year-olds mean incidence decreased from 174 (1996-2001) to 112 (2002-2010).
In persons = 10 years, mean incidence increased from 15 (1996-2004) to 33 (2005-2010). Likewise, immunosurveillance data showed an increase of pertussis-specific antibodies from 4% (1996-1997) to 9% (2006-2007).
Mean vaccine-effectiveness in 1-3-year-olds increased from 31% (1996-2004) to 81% (2005-2010).
In the cohorts targeted for the booster vaccination vaccine-effectiveness remained high (mean 64%) with still 28% in the first two cohorts vaccinated, i.e. 8-9 years after introduction of vaccination.
Conclusion: Due to changes in the vaccination program, the incidence of pertussis is decreasing in children. Vaccine-effectiveness considerably improved after introduction of an acellular vaccine. More than 5 years after its implementation, vaccinated cohorts still benefit from the introduction of a preschool booster dose.
In contrast, the incidence of pertussis in adults is increasing, probably due to increased circulation following pathogen adaptation. Further optimization of the vaccination strategy should be addressed.

P023

Preventive microbiological interventions to control two concurrent outbreaks of multidrug-resistant *Klebsiella pneumoniae* – making the iceberg visible

R.H.E. van Oosterhout, J.P. Arends, L.J.W. ten Horn, J.A.C. van der Weerd, M. Ciccolini, D.M. Borst, G.A. Kampinga, H.J. Schotsman, M. Lokate, J.C. Rahamat-Langendoen, J.W.A. Rossen, A.W. Friedrich
University Medical Center Groningen, Dept. of Medical Microbiology, Groningen

Introduction: Early detection of unidentified colonized patients is a key factor in controlling an outbreak of multidrug-resistant gram-negative pathogens. We describe strategies of microbiological screening as part of the outbreak management to control two concurrent outbreaks of extended spectrum bèta-lactamase (ESBL) producing *Klebsiella pneumoniae* in a Dutch university hospital.
Methods: At the time of outbreak alert, 6 patients were identified to be infected with ESBL producing *K. pneumoniae* on 2 different wards. Immediate molecular typing (Diversilab) revealed that each ward had a different cluster of multidrug-resistant ESBL producing *K. pneumoniae*. We expected in total 15 cases based on the maximal attack rate of *K. pneumoniae* described in literature (iceberg estimation). In addition to the extended infection control precautions, 3 strategies of microbiological screening were applied; (A) all 42 patients present on the two affected wards at the time of closure, were twice weekly screened; (B) all 90 high-risk patients (former room or ward mates of confirmed cases) were screened at least twice using home-sending packages and; (C) 154 patients on 10 high risk wards were screened once. High risk wards were defined as the wards with the largest number of patients transferred from one of the outbreak wards and were identified using network analysis. To search for historical cases since January 2012, isolates of 34 patients with ESBL producing *K. pneumoniae* with similar resistance patterns were typed.
Results: In total, 438 samples of 286 patients were collected. Out of these, another 5 cases were identified; 3 patients using strategy A and 2 patients using strategy B. We identified one more probable case using strategy B, however this was not confirmed using Diversilab typing. No new cases were detected using strategy C. No patients became infected or colonized after the implementation of the infection control precautions. Molecular typing of historical isolates identified 3 more cases, all belonging to one of the two clusters. In total, 8 out of 14 confirmed cases (= a manifestation index (MI) of 55% in cluster 1 and MI of 60% in cluster 2) had clinical signs of infection. The transmission rate was 8.2% for cluster 1 (5/32) and 15.6% (9/109) for cluster 2. The main (presumed) transmission route was (in)direct patient contact under antibiotic selective pressure.
Conclusion: Several recommendations can be proposed based on the results of these outbreaks. (1) Contact isolation in a single room seems to be essential for all patients infected or colonized with ESBL producing *K. pneumoniae* since 13 out of 14 cases shared rooms or toilet facilities; (2) Screening of roommates after one unexpected finding is necessary in the early control of an outbreak; (3) Next to a continuous ESBL surveillance system, microbiological screening and molecular typing are essential to detect nosocomial transmission in an early stage; (4) Antibiotic stewardship is crucial and; (5) Network analysis was valuable in the risk assessment, helping to focus infection control interventions during the outbreaks. In conclusion, the combination of microbiological interventions and extended infection prevention precautions were successful in stopping the outbreaks immediately.

P024

Bioterrorism in patient diagnostics

M.C. de Vries, D.W. Notermans, F.A.G. Reubsaet
RIVM, IDS-bacteriology, Bilthoven

The anthrax-letters in the USA in 2001 raised awareness in bioterrorism. CDC (Centre for Disease Control) made a list of potential agents to increase clinical and diagnostic alertness and preparation. This list includes bacteria like *Bacillus anthracis, Burkholderia mallei, B. pseudomallei, Francisella tularensis, Yersinia pestis, Brucella* ssp. and *Coxiella burnetii*. These agents, almost all zoonotic, are endemic in different parts of the world, and occur occasionally as imported infections in the Netherlands. Intentional release is less frequent than natural incidence, despite the rare occurrence of most of the infections in the Netherlands. Coxiella is an exception as it is endemic in the Netherlands causing a large outbreak between 2007 and 2010.
When one of those bacteria is identified, the question is how to act and how to distinguish a natural infection from a bioterroristic attack.
Confirmation of the result by a swift and highly specific method is essential to act appropriate to the situation or prevent unnecessary actions and panic. Since most of those organisms are uncommon in the Netherlands and not all laboratories possess the necessary tests, confir-

mation methods, including real time PCR, are available at the LIS-RIVM. When suspicions or earlier results are not confirmed, sequence determination of the 16S-gene and, when possible, biochemical techniques are used to identify the pathogen.
However, as soon as the result is confirmed, further information about this patient and other cases can specify the source of infection. When the patient has been travelling in an endemic region during the incubation period, this is a likely source of infection. In addition, habits like intravenous drug-use can relate an antrax infection to the current European outbreak among drug-users. Furthermore, the symptoms of the patient can indicate an (un)common route of infection.
Simultaneously, an inventory about other cases needs to be made. Using national E-mail alert services such as labinf@ct or *'het signaleringsoverleg'*, other laboratory can be informed about this unexpected strain and its specific diagnostics. Increase of awareness will point out possible other cases. However, to distinguish a single case from the background incidence, the latter must be known or at least estimated. Combining the number of cases with the (estimated) background incidence demonstrates the likeliness of the event occurring naturally.
Altogether, confirmation of these potential bioterroristic bacteria in the Netherlands is achieved by using a validated test available at the LIS-RIVM, which are practised regularly for example during external quality assessments. The number of expected cases indicates the amount of chemicals, kits, oligonucleotides, and trained personnel required in case of an outbreak.
With an actual case, patient information and information about other cases can add up to the source of infection and so to the human involvement in the release.
Based on a case study about a Dutch *F. tularensis* case, the important steps to follow in patient diagnostics to define and act in case of deliberate release of pathogens will be explained. The focus will be on the role of the medical microbiological laboratory and LIS-RIVM.

P025

Iron restriction-induced adaptations in the wall proteome of the opportunistic fungal pathogen *Candida albicans*

F.M. Klis, A.G. Sorgo, C.J. Heilmann, S. Brul, C.G. de Koster, L.J. de Koning
University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam

Introduction: *C. albicans* is an opportunistic fungal pathogen that can grow as yeast cells and form pseudohyphae and hyphae. Its walls consist of an internal skeletal layer surrounded by a coat of covalently linked, outwardly extending glycoproteins. Iron availability in a mammalian host is generally extremely limited and severely restricts growth of invading micro-organisms (a form of nutritional immunity'). Under low-iron conditions *C. albicans* uses three iron uptake mechanisms. (1) The siderophore pathway. *C. albicans* cannot synthesize siderophores itself, but it can take up foreign siderophores. (2) The reductive pathway, the first step of which is carried out by plasma membrane-located ferric reductases. (3) The Rbt5 pathway. Rbt5 is the most prominent member of a family of surface-located proteins, which consists of Rbt5, Csa1, Pga7, and Pga10. Rbt5 has been shown to strongly promote growth of *C. albicans* provided with heme or hemoglobin as the sole iron source. Furthermore, Rbt5, Csa1, and Pga10 can bind heme. All family members contain at least one CFEM domain (Common in several fungal etracellular membrane cproteins), which is characterized by a conserved 8-cysteine pattern. Here we show that in response to iron restriction the wall levels of the Rbt5 family members strongly increase and that the heme-binding activity of the Rbt5 family members probably maps to their CFEM domain.
Methods: We used Fourier transform mass spectrometry to identify and quantify the changes in the wall proteome in response to iron restriction. A fixed amount of ^{15}N-metabolically labeled cell walls was included as an internal standard in each experiment. This produced ^{14}N/^{15}N isotopic ratios (light/heavy; query/internal standard) for individual wall proteins and allowed comparison between growth conditions.
Results: Addition of the membrane-impermeable ferrous iron chelator bathophenanthroline disulfonic acid to the rich growth medium YPS (Yeast extract-Peptone-Sucrose) or use of the synthetic medium YNBS (Yeast Nitrogen Base-Sucrose) buffered at pH 7.4 strongly decreased the iron content of the cells. This was accompanied by a sharp rise in the wall levels of Rbt5, Csa1, Pga7, and Pga10. Secretion in the medium of the related, CFEM-domain protein Csa2 strongly increased as well. In addition, the wall levels of Als3 (another iron-acquisition protein that binds ferritin), the *adhesin* Als4, and the two hypha-associated proteins Hyr1 and Rbt1 increased. Finally, the mass spectrometric spectra of tryptic peptides originating from the CFEM domain of Rbt5 family members revealed that during electrospray ionization iron adducts were formed. This demonstrates that CFEM peptides are capable of tightly binding iron *in vitro*, and probably also *in vivo*.
Conclusion: 1. Upon iron restriction the levels of iron-acquisition wall proteins strongly increase.
2. The heme-binding activity of Rbt5 family members maps to the CFEM domain. This probably involves their cysteine residues.
3. We propose that wall-bound Rbt5 family proteins are involved in iron acquisition. Conceivably, they form a similar heme relay system as found in gram-positive bacteria.
4. Fungal CFEM proteins widely occur among ascomycetous fungi. This raises the question of whether they are generally involved in iron acquisition.

P026

Modeling antibiotic resistance of gram-negative bacteria during selective digestive tract Decontamination in intensive care units

E.A.N. Oostdijk, M.C. Bootsma, A.M.G.A. de Smet, M.J.M. Bonten
University Medical Center Utrecht, Medical Microbiology, Utrecht

Introduction: Selective digestive tract decontamination (SDD) is a prophylactic antibiotic regimen consisting of topical antibiotics (tobramycin (TOB), colistin (COL) and amphotericin B) applied in oropharynx and intestinal tract throughout intensive care unit (ICU)-stay, combined with a 4-day course of cefotaxime (CTX). SDD exerts continuous antibiotic pressure of TOB and COL and was associated with 87% increase in cephalosporin use (compared to standard care (SC)) (de Smet NEJM 2009). Yet, in Dutch ICUs SDD was also associated with 38% lower acquisition rates of antibiotic resistance of gram-negative bacteria (AR-GNB). Using data from this study we investigated the dynamical interactions between antibiotic pressure (systemic and topical antibiotics) and admission rates of AR-GNB using a mathematical model.
Methods: 1911 patients had at least 1 rectal culture result and the admission and acquisition rate of AR-GNB was determined for TOB+COL, CTX and TOB+COL+CTX. Parameters of the model were estimated by MCMC-simulations using uninformative priors. All available data on duration of stay with corresponding culture dates and results were used at an individual patient level. Posterior parameter estimates were applied to the same model without SDD antibiotic pressure (a1), without decontamination (?) and with a 47% reduction in CTX antibiotic pressure (a2). Sensitivity analyses were performed by adding various parameters of cross-transmission to the model.
Results: 102 patients were colonized with AR-GNB (93 CTX, 5 TOB+COLand 6 TOB+COL+CTX). Median and 95% credibility intervals for overall resistance prevalence were 3.2% (2.6-4.0) during SDD and 9.2% (9.1-9.4) during SC. If the admission prevalence would increase 5-fold, mimicking settings with high endemicity of antibiotic resistance, overall resistance would be 12.7% and 24.5% during SDD and 26.4% and 50.0% during SC. Adding cross-transmission as a separate parameter to the model, resulted in overall resistance rates of 15% and 39% in low endemic setting for SDD and SC respectively and of 56% and 78% respectively in high endemic settings. Increasing the importance of cross-transmission, resulted in higher rates of overall resistance and smaller differences between SDD and SC.
Conclusion: The model accurately reflects the observed beneficial effects of SDD on antibiotic resistance in Dutch ICUs, as compared to SC. The model also demonstrates that the beneficial effects remain with higher admission prevalence. Cross transmission reduces the beneficial effects, but SDD still outperforms SC.

P027

In vitro and *in vivo* imaging of human respiratory syncytial virus infections using recombinant viruses expressing enhanced green fluorescent protein

D.T. Nguyen[1], K. Lemon[2], M. Ludlow[3], S. Yüksel[1], S. McQuaid[2], L. Rennick[3], A.D.M.E. Osterhaus[1], W.P. Duprex[3], R.L. de Swart[1]
[1]Erasmus MC, Viroscience, Rotterdam, [2]Queen's University of Belfast, Microbiology, Belfast, Ireland, [3]Boston University, Microbiology, Boston, USA

Introduction: Human respiratory syncytial virus (HRSV) is an important cause of acute respiratory tract disease in infants, immunocompromised subjects and the elderly. Although HRSV infection usually induces relatively mild and self-limiting upper respiratory tract infections, in some cases (usually estimated as 1-2%) the virus spreads to the lower respiratory tract and may cause severe bronchiolitis or pneumonia. A substantial proportion of these patients require hospitalization, and occasionally mechanical ventilation. Despite more than half a century of research there is no licensed vaccine available. Studies into viral pathogenesis require clinical isolates of known provenance and passaged in disease-relevant cell lines. In this study we have used a recombinant HRSV strain expressing enhanced green fluorescent protein (EGFP) to explore its pathogenicity *in vitro* and *in vivo*.
Methods: We have used a new molecular clone based on RNA isolated directly from the nose of a human patient infected with HRSV subgroup B (rHRSV^{Bos}) expressing EGFP from an additional transcription unit (rHRSV^{Bos}EGFP). The virus was compared with rgRSV, a GFP-expressing molecular clone of the laboratory-adapted HRSV strain A2. The human larynx carcinoma cell line HEp-2 was used to grow low passage virus stocks. Infections of CHO-K1 and the glycosaminoglycan-deficient CHO-A745 cells were used to determine the GAG index' as described by Hallak et al. (2007). The viruses were used to infect primary normal human bronchial epithelial (NHBE) cells grown in inserts and differentiated at air-liquid interphase. These cells provide a bridge between *in vitro* and *in vivo* infection models. Subsequently, cotton rats (*Sigmodon hispidus*), the most susceptible small animal

model for HRSV, were intranasally infected with a low dose (10^4 $TCID_{50}$) virus. Nasal septum, nasal conchae, trachea, and agarose-inflated lung slices were imaged.
Results: GAG-indices for $rHRSV^{Bo5}$, $rHRSV^{Bo5}$EGFP and rgRSV were 1.93, 2.64 and 13.4, respectively. Infection with $rHRSV^{Bo5}$EGFP in well-differentiated NHBE showed a more effective apical infection of ciliated epithelial cells than the laboratory adapted rgRSV, as evidenced by enumeration of $EGFP^+$ cells. $rHRSV^{Bo5}$EGFP was also more pathogenic in cotton rats *in vivo* than rgRSV. EGFP-positive cells were observed in the nasal septum, nasal conchae, trachea and in the lungs, where they were mainly detected in the bronchial and bronchiolar epithelium.
Conclusion: In this study, molecular clones of non-tissue-cuture-adapted HRSV subgroup B ($rHRSV^{Bo5}$), either or not expressing EGFP ($rHRSV^{Bo5}$EGFP), were evaluated *in vitro* and *in vivo*. Compared to rgRSV the new clones proved to be more virulent in NHBE cells and in cotton rats. Attaining a comprehensive understanding of the processes involved in cell attachment and penetration of this wild-type rHRSV strain will facilitate pathogenesis and intervention studies and aid high-throughput antiviral screening.

P028
Revised Dutch guideline infection prevention 'Highly resistant micro organisms'

B.M. Roede[1], J. Kluytmans[2], R. Hendrix[3], I. Willemsen[4], T.H. Daha[1], M.A. Leverstein-van Hall[5], J.H. van Zeijl[6]
[1]*Working Party on Infection Prevention, LUMC, Leiden,* [2]*Amphia Hospital, Breda and VU University Medical Center, Amsterdam,* [3]*Laboratory of Medical Microbiology Twente Achterhoek, Enschede, Enschede,* [4]*Amphia Hospital, Breda,* [5]*Bronovo Hospital, The Hague,* [6]*Izore Center for Infectious Diseases Friesland, Leeuwarden*

Introduction: The Dutch Working party on Infection Prevention develops national guidelines for healthcare facilities, primarily hospitals. They are considered as professional standards. Recently, the guideline Highly Resistant Micro Organisms (HRMO)' was updated.
Methods: Guidelines are developed following a standardized procedure, with experts playing a central role. The Dutch Societies for Medical Microbiology, for Infectious Diseases, and for Hygiene and Infection Prevention are always represented. Recommendations are based on literature when available, and on expert opinion.
Results: Compared with the former guideline, we simplified recommendations to improve compliance in daily practice. We abolished distinction between intensive care and regular nursing wards for defining HRMO and for screening of contact patients; brought together '*Escherichia coli, Klebsiella* species and other *Enterobacteriaceae*' to one category '*Enterobacteriaceae*', where trimethoprim/sulfamethoxazole resistance was removed as criterion for HRMO. The same holds for ceftazidim resistance in *Acinetobacter* species. For patients nursed in a foreign hospital, the additions 'or have been for surgery, or have got a drain, etc.' were removed. Other recommendations were strengthened. Patients with Carbapenem Producing *Enterobeacteriaceae* (CPE) were specifically defined to be nursed in a single room. After discharge of a patient with HRMO, disinfection of the room is required. In case of outbreak and transmission, patients should be nursed in strict isolation whenever possible.
HRMO were defined as extended spectrum bèta-lactamase (ESBL) or carbapenemase (CP) producing *Enterobacteriaceae*, or strains resistant to fluorochinolones (FQ) and aminoglycosides (AG); CP producing *Acinetobacter* species, or strains resistant to FQ and AG; *Stenotrophomonas maltophilia* resistant to trimethoprim/sulfamethoxazole; *Pseudomonas aeruginosa* resistant to three specified groups of antibiotics including carbapenems; *Streptococcus pneumoniae* resistant to penicillin and/or vancomycin, and *Enterococcus faecium* resistant to both amoxicillin and vancomycin.
Admitted patients should be tested for presence of HRMO if they were admitted to a foreign hospital less than two months ago for at least 24 hours, or if they came from any ward with an ongoing outbreak with HRMO. They are placed in contact isolation awaiting test results.
Individual cases with proven HRMO are always nursed in contact isolation, for *Acinetobacter* strict isolation is required. For HRMO positive patients visiting the outpatients' department, no additional prevention measures are required. Contact patients are screened following the ring' principle.
In case of an outbreak, patients need to be nursed in a single room or in cohort. When the outbreak proceeds, the isolation measures need to be extended to strict isolation if possible. Molecular typing is needed to confirm relationships between strains or plasmids. Screening of contact patients is required for all patients.
Conclusion: This guideline updates infection control measures for policy making in hospitals in order to prevent the transmission of HRMO, for which is an urgent request considering current situations. How long patients with HRMO should be considered positive, implying infection control measures, and whether a patient follow-up system for HRMO should be recommended, is still under discussion.

P029
A diagnostic screening algorithm to asses the public health risk of STEC

R.F. de Boer[1], A. Ott[1], H.R. Scheper[1], G.J. Wisselink[1], J.W. Rossen[2], A.M.D. Kooistra-Smid[1]
[1]*Laboratory for Infectious Diseases, Research and Development, Groningen,* [2]*UMCG, Medical Microbiology, Groningen*

Objectives: Shiga toxin-producing *Escherichia coli* (STEC) poses a serious public health concern because of its ability to cause outbreaks, hemorrhagic colitis (HC), and hemolytic-uremic syndrome (HUS). In particular, STEC strains belonging to serogroups O26, O103, O111, O121, O145 and O157 are known causative agents of outbreaks and serious illness. This study describes a rapid screening algorithm to discriminate STEC infections associated with public health risk from less virulent ones.
Methods: A total of 2052 stool samples from patients with presumed infectious gastro-enteritis were prospectively screened using real-time multiplex PCR (mPCR) targeting stx1/stx2. Subsequently, PCR positive stool specimens were cultured (SMAC) and enriched (> 16 hrs) in BRILA broth, followed by culture on CHROMagar STEC medium. Simultaneously, enriched BRILA broth was used for DNA isolation. Confirmatory real-time mPCRs were performed targeting stx1/stx2/escV/bfpA/aggR/aat/O26/O103/O104/O111/O121/O145/O157. Furthermore, STEC suspected CHROMagar colonies (n = 5 per sample) were tested with mPCRs. PCR results were used for presumptive seropathotype (SPT) classification and to assess the presence of viable STEC. Furthermore, a DNA micro-array was used to determine the virulence gene profile of culture isolates.
Results: The detection frequency of stx1/stx2 genes was 2.3% (n = 47). The algorithm was applied to 42 STEC PCR positive samples (40 patients). Further analysis of the enriched BRILA broth samples identified SPT A (n = 1, 2%), SPT B (n = 4, 10%), SPT C (n = 8, 19%), and SPT D (n = 25, 60%), EPEC (n = 1, 2%) and EAEC (n = 1, 2%). In 4 samples no viable STEC could be detected.
A total of 7 mPCR positive samples (17%) were CHROMagar STEC culture positive: SPT A (n = 1; O157), SPT B (n = 3; 2x O26, O145), SPT C (n = 2), SPT D (n = 1). The latter three were O-type PCR negative. Only one mPCR positive sample was SMAC positive: SPT A (O157).
The delta stx1/stx2 CT values of enriched vs direct samples ($CT_{enriched} - CT_{direct}$) ranged from +5 to -21. In 32 (76%) enriched samples the delta CT value was ≤ 0.
Conclusion: The diagnostic algorithm enables fast discrimination of virulent STEC associated with outbreaks (SPT A and B) and HUS (SPT A, B and C) from less virulent STEC (SPT D).
Compared to routine SMAC, the CHROMagar STEC improved the culture yield with additional identification of non-O157 isolates. The algorithm enables the detection of viable STEC in stool samples.

P030
Mumps outbreak in the Netherlands, 2010-2012

S. Gouma, R. van Binnendijk, D. Gijselaar, J. Cremer, H. Boot, S. Hahné, M. Koopmans
RIVM, Virology, Bilthoven

Introduction: Since January 2010, a mumps outbreak has been going on in the Netherlands. To date (January 2013), 1579 cases have been reported. Most patients were young adults who were vaccinated against mumps during childhood. Preliminary characterization of strains from patients in different regions of the country showed a small but significant genetic diversity among circulating strains. Here we describe this genetic diversity for patients diagnosed between 2009 and 2012, by sequencing both the SH gene, which is genetically the most diverse, and the HN gene, which codes for the surface glycoprotein involved in antibody binding. We looked for possible correlations between sequence types and epidemiological, clinical and virological data.
Methods: Between January 2010 and September 2012, 795 mumps PCR positive specimens were used for phylogenetic analysis of the 316 bp fragment encoding the SH protein and a representative set of samples were also sequenced for the entire HN gene (1749 bp, preliminary data). Salivary viral load was determined by realtime PCR (n = 290).
Results: At the beginning of the outbreak in the Netherlands in January 2010, the major mumps genotype detected was G5, with an SH sequence identical to the one that caused a major outbreak among the Jewish community in New York in 2009 (G5-NY). In March 2010, a first variant of this G5 was detected (G5 Groningen). This variant differs two nucleotides in the SH sequence compared with G5-NY, resulting in one silent and one missense mutation. Throughout the mumps outbreak, G5 Groningen became the most dominant strain found in the Netherlands (n = 538), compared to the first outbreak variant (n = 187). A few other G5-subtypes have been detected, but only during a short period of time and restricted to 1-2 mutations in the SH gene compared to G5-NY and G5 Groningen. 20 patients had genotypes not belonging to the G5 strain. Contrary to the SH sequences, preliminary data show that phylogenetic clusters formed by HN sequences differ from clusters based on SH sequences. A first group-wise analysis of the viral loads showed that persons infected with G5 Groningen had higher salivary viral loads when compared to individuals infected with the outbreak strain. In general, viral load was determined by time between sampling, onset of the disease and vaccination history, but these factors had no effect on the difference in viral load observed between the two G5 subtypes.

Conclusion: Based on SH gene sequences, the first Dutch outbreak strain in 2010 appears to be identical to the G5 New York strain, whereas G5 Groningen, which is the most dominant mumps strain in the more recent outbreaks, has two mutations in the SH gene. Strikingly, salivary viral loads are higher in patients infected with G5 Groningen when compared to the outbreak strain, which suggests a virological advantage of this variant in the transmission of mumps virus. Whether these differences are caused by certain mutations in the viral genome needs to be determined.

P031

Population structure of *Pseudomonas aeruginosa* and the prevalence of epidemic clones in patients with cystic fibrosis over four years.

R. van Mansfeld[1], A.M.M. de Vrankrijker[1], R. Brimicombe[2], C. van der Ent[1], T. Wolfs[1], M.J.M. Bonten[1], R. Willems[1]
[1]UMC Utrecht, Medical Microbiology, Utrecht, [2]Haga Teaching hospital, Medical Microbiology, The Hague

In a previous cross-sectional study in 2007, we investigated the population structure of *Pseudomonas aeruginosa* (PA) in cystic fibrosis (CF) patients in the Netherlands. PA carriage in the respiratory tract of all CF patients visiting two major CF centres was determined by standard microbiological culture techniques, and all obtained phenotypically different isolates were genotyped with MLST. 596 patients were included, representing 46% of the Dutch CF population. Of 265 PA positive patients 60% harboured a strain that was also found in at least 2 other patients. ST406 was found in 15% of the patients colonised with PA and more frequently among patients 15-25 yrs of age. ST406 is not genetically linked to other international epidemic clones.
In this follow-up 2011 study we investigated the effects of segregating CF patients (which started in 2006) on the population structure of PA in CF patients and persistence of STs, using identical methodology as in the previous study.
Respiratory tract samples were obtained from 627 (> 96%) CF patients visiting either hospital. PA was detected in 51% of the patients studied and from 276 patients with PA (86%), 427 isolates were typed with MLST. This yielded 145 STs. 164 PA positive patients were included in both studies. In 113 patients (40%) multiple (2-4) phenotypic different isolates were isolated. In 8% the phenotypic different isolates actually represented different STs. This was similar to findings in 2007 (10%). 22 STs were shared between more than two patients and were found in 50% of patients (60% in 2007). ST406, the highly prevalent Dutch clone, was found in 14% of patients (15% in 2007). The age distribution of ST406 carriers was shifted to an older age compared to 2007 (20-30 yrs).
In > 90% of the patients that were included in both studies genotypes persisted. The Simpson diversity index (DI) of the PA population was 97.87 (95% confidence interval (CI): 97.07-98.67) in 2011 and 97.3 (CI 96.2-98.4) in 2007.
The combined data of two cross-sectional studies in 2007 and 2011 demonstrate that the population structure of PA is highly diverse with > 90% persistence of strains in individual patients during four years. The previously observed association between age and presence of ST406 seems a cohort phenomenon rather than age-related niche adaptation.

P032

Correlations between peripheral blood bacterial DNA load, interleukin-6 levels and C-reactive protein levels in acute Q fever

M. Kremers[1], R. Janssen[2], L.M. Kampschreur[3], P.M. Schneeberger[4], P.M. Netten[1], A. de Klerk[2], H. Hodemaekers[2], M.H. Hermans[5], D.W. Notermans[2], P.C. Wever[4]
[1]Jeroen Bosch Hospital, Dept. of Internal Medicine, 's-Hertogenbosch, [2]National Institute for Public Health and the Environment, Bilthoven, [3]University Medical Center Utrecht, Division of Medicine, Deptartment of Internal Medicine and Infectious Diseases, Utrecht, [4]Jeroen Bosch Hospital, Dept. of Medical Microbiology and Infection Control, 's-Hertogenbosch, [5]Jeroen Bosch Hospital Dept. of Molecular Diagnostics, 's-Hertogenbosch

Introduction: From 2007 to 2010, the Netherlands experienced the largest outbreak of acute Q fever reported to date with over 4000 notified cases. We have previously shown that C-reactive protein (CRP) is the only traditional infection marker adequately reflecting disease activity in acute Q fever. Interleukin-6 (IL-6) is the principal inducer of CRP. We questioned whether increased CRP levels in acute Q fever patients coincide with increased IL-6 levels and if these levels correlate with the bacterial DNA load in serum.
Methods: We analysed 102 patients diagnosed with seronegative polymerase chain reaction (PCR) positive acute Q fever from April to August 2009. Seronegativity was defined as the absence of IgM antibodies against *Coxiella burnetii* phase II antigens, measured by either immunofluorescence assay (before 1 May) or enzyme-linked immunosorbent assay (ELISA). PCR positivity was defined as the presence of *C. burnetii* DNA in serum (cycle threshold (Ct) value < 45) as measured by an in-house real-time PCR. Ct value was considered a semiquantitative representation of *C. burnetii* DNA load, with lower Ct value representing higher load. Serum samples stored at -20 °C were thawn and subsequently IL-6 and CRP levels were analysed by polysterene bead-based Luminex assay and ELISA, respectively.
Results: Statistical analysis using the Spearman's rank coefficient showed a negative correlation between Ct values and IL-6 levels (r_s = -0.283, p = 0.004). The same test also revealed a negative correlation between Ct values and CRP levels (r_s = -0.229, p = 0.021). Although the effect sizes of these correlations were weak, they were both statistically significant. Furthermore, a statistically significant positive correlation between IL-6 and CRP levels was demonstrated (r_s = 0.641, p < 0.001). The effect size of this relationship was strong.
Conclusion: Our findings revealed a negative correlation between serum Ct values and IL-6 as well as CRP levels in acute Q fever patients. Furthermore, we showed a strong positive correlation between the levels of IL-6 and CRP in these patients. These observations suggest that the bacterial DNA load level directly influences production of IL-6, which in turn directly induces production of CRP. Additional research is necessary to determine whether a higher bacterial DNA load relates to a more severe clinical disease course.

P033

An *in vitro* burn wound model to test topical treatments against *Pseudomonas aeruginosa*

B.K.H.L. Boekema[1], L. Pool[1], R. Loef[1], E. Middelkoop[2], M.M.W. Ulrich[2]
[1]VSBN, Preklinisch onderzoek, Beverwijk, [2]VU University Medical Center, Plastic, Reconstructive and Hand Surgery, Amsterdam

Introduction: A constant threat in burn wound care is bacterial contamination. These contaminations, particularly those with *Pseudomonas aeruginosa*, have to be treated to prevent the spread to other tissues which can result in infection. Use of systemic antibiotics is limited because of the low penetration rate into dead tissue and the risk of resistance development. Topical (local) treatments with good bactericidal effects are available but can have detrimental effects on the healing process, e.g. silver based products increase healing time. Therefore bactericidal topicals, which do not impede wound healing are needed. Here we present an in vitro model in which both aspects can be tested.
Methods: We evaluated the bactericidal and cytotoxic effects of a honey based gel (l-Mesitran Soft) and silver sulphadiazine (flammazine and plurogel) in an *in vitro* human burn wound model with *P. aeruginosa*. Controls consisted of vaseline, cetomacrogol, an alginate gel or no treatment. Skin was obtained form 30 donors in total. Topical treatments were applied on the burn wound models after adding *P. aeruginosa*. At different time points, bacteria were dislodged and counted by plating dilutions. Cytotoxic effects (re-epithelialization and proliferation) were evaluated histologically in samples of burn wound models treated topically for 3 weeks, without bacteria.
Results: l-Mesitran significantly reduced the bacterial load (5-log reduction) up to 24 h but did not completely eliminate bacteria from the burn wounds. After Flammazine treatment, only a few colony forming units were observed at all time points. Plurogel treatment greatly reduced the bacterial load (6.8-log reduction). In contrast, reepithelialization of the wound was significantly reduced after application of flammazine compared to l-Mesitran or control and was even absent after plurogel treatment.
Conclusion: This *in vitro* model of burn wound infection can be used to evaluate topical treatments for bactericidal activity and effect on wound healing.
Silver based products strongly reduce bacterial load but also reduce wound healing.
l-Mesitran is a good alternative for treating burn wounds but the slightly lower bactericidal activity in the burn wound model warrants a higher frequency of application.

P034

Comparing *Borrelia* OspA, IGS and FlaB multiplex and 16S cDNA PCR

H.C.G.M. de Leeuw[1], L. Hollemans- van Dijk[1], H. Sprong[2], A.H. Brandenburg[3], J.W.R. Hovius[4], M. Riemens[5], A. Breugelmans[5], P.J. Westenend[5], J.G. Kusters[6]
[1]RLM-PAL, Molecular diagnosis, Dordrecht, [2]RIVM, Laboratory for Zoonoses and Environment, Bilthoven, [3]IZORE, Center Infectious Diseases, Leeuwarden, [4]UvA, Center for Experimental and Molecular Me, Amsterdam, [5]Paldordt, Dordrecht, [6]UMCU, Dept. of Medical Microbiology, Utrecht

Introduction: *Borrelia* infection after a tick bite can cause Lyme disease. Diagnosis of active infection is problematic, because PCR even on liquor samples is regarded not sensitive enough to detect all Lyme serology positive samples. On the other hand serology may remain positive after infection has cleared, so not all serology positive samples may need treatment. In order to increase sensitivity of the PCR on liquor two methods were compared.
Methods: Real time PCR was performed on DNA isolates of patient samples of known serology and on dilution series of Borrelia strains B31, PKO and PBI using a multiplex PCR, including target genes OspA, IGS and FlaB. Also a cDNA PCR for 16S rRNA was tested (Ornstein et al, 2006).
Results: Average Ct values for the different target genes of the multiplex were not significantly different (ANOVA, Bonferroni). Multiplex PCR showed only sporadic extra positive samples (1 of 30 serology positive liquor samples) besides those that were OspA positive in retrospective patient material. All sequenced PCR products from non OspA positive samples had highest homology to *Borrelia*. The 16S cDNA PCR had a significantly lower Ct compared to 16S DNA PCR on *Borrelia* strain dilutions and showed 100% positivity in higher dilutions. In spite of the fact

that this 16S cDNA did not have a significantly different Ct compared to OspA DNA PCR on the dilution series of the three strains taken together, the 16S cDNA PCR remained positive in 100% of PCRs per tested sample in at least 4 further dilution steps (1:1) compared to OspA DNA PCR on the combined strains tested (together 16 times further diluted). Retrospective patient samples did not show increased positivity for the 16S cDNA PCR. However, we could not prove any effect of the RT reaction in the samples tested. Based on the frequency of positivity the number of bacteria present in different patient samples was estimated as 2 bacteria per PCR (40 bacteria per lysate) for the positive punctate samples, less than 1 bacterium per PCR (20 bacteria per lysate) for liquor samples and for tick DNA 16 bacteria per PCR. Doubling the amount of input material (not possible for all specimens) increased the positivity frequency in Borrelia positive liquor samples from 21% to 44% PCR positivity.

Conclusion: 1. The 16S cDNA PCR is technically more suited to detect low levels of *Borrelia* compared to the OspA PCR in cultured *Borrelia* strain dilutions.

2. Retrospective sample material left over after serology did not show sufficient RNA quality to determine whether 16S cDNA PCR might increase sensitivity of PCR in clinical specimins over OspA PCR.

3. Using multiple target PCR, due to the low frequency of PCR positivity in *Borrelia* positive clinical samples, more positive samples can be identified with higher certainty in retrospective liquor material compared to OspA PCR alone, but this may not lead to great difference in percentage of positive patient samples compared to input optimized OspA single target PCR.

P035

Q fever vaccination leads to limited immune response in elderly people with risk factors for chronic Q fever

T. Schoffelen[1], T. Herremans[2], T. Sprong[3], P.C. Wever[4], M. Nabuurs-Franssen[3], H. Bijlmer[2], M. van Deuren[1]

[1]*Radboud University Nijmegen Medical Centre, Dept. of Medicine, Nijmegen,* [2]*National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Bilthoven,* [3]*Canisius Wilhelmina Hospital, Dept. of Internal Medicine & Medical Microbiology, Nijmegen,* [4]*Jeroen Bosch Hospital, Dept. of Medical Microbiology & Infection Control, 's-Hertogenbosch*

Introduction: At the end of the Dutch Q fever outbreak, people at risk for chronic Q fever (with cardiac valvular or vascular disease) were offered vaccination against *Coxiella burnetii* with the Australian inactivated whole cell vaccine Q-vax. Previous reports describe high efficacy of the vaccine in young abattoir workers and healthy volunteers. However, no data are available for elderly people with a risk factor for chronic Q fever. Here, we report on the immune response to Q fever vaccination in this particular population and compare it with the immune response after natural Q fever infection.

Methods: In total, 263 vaccinees (median age 66 yrs), all serologically and skin test negative in pre-vaccination screening, were studied prior to vaccination and 6 and 12 months after vaccination. Humoral immune response was assessed by immunofluorescence assay; seropositivity was defined as any IgG or IgM anti-*C. burnetii* titre ≥ 1:32, regardless of anti-phase I or phase II antibody type. Cellular immune response was assessed by measuring *C. burnetii*-specific *in vitro* interferon-gamma (IFN-g) production in whole blood (i.e. IFN-g test'). IFN-g test was considered positive when production was ≥ 32 pg/ml. A cohort of 200 patients, diagnosed with acute Q fever during the Dutch Q fever outbreak and followed up after 6 and 12 months, served as control group.

Results: Prior to vaccination, 200 vaccinees had a negative IFN-g test, 42 a positive IFN-g test, while 21 had inconclusive IFN-g test results. In the 200 naive vaccinees, 40.1% became seropositive and 61.7% had a positive IFN-g test after 6 months; 28.0% were positive in both tests. Anti-phase I IgG was detected in 33.3% and anti-phase II IgG in 27.4%. IgM, either anti-phase I or anti-phase II, was present in only a minority (9.1%). After 12 months, 56.5% of naive vaccinees were seropositive and 56.4% had a positive IFN-g test.

The 42 vaccinees with a positive IFN-g test prior to vaccination showed a higher seroconversion rate than the naive vaccinees: 73.8% at 6 months, and 83.3% at 12 months. In 200 control individuals (median age 58 yrs), followed up after a natural Q fever infection, 86.0% had anti-phase I IgG after 6 months and 75.5% after 12 months, while 99.5% remained seropositive for anti-phase II IgG over the whole year.

Conclusion: The immune response after Q fever vaccination, expressed as percentage seropositivity after 6 and 12 months, in an elderly population with risk factors for chronic Q fever is less than previously reported for vaccinated healthy abattoir workers and less than in naturally infected individuals.

Vaccinees with a positive *C. burnetii*-specific IFN-g test prior to vaccination had a higher seroconversion rate. Although cross-reactivity cannot be excluded, this suggests boosting of pre-existing specific anti-*C. burnetii* immunity that was missed by serology or skin testing but detected by the IFN-g test prior to vaccination.

P036

High resolution typing of livestock-associated methicillin-resistant *Staph*ylococcus aureus using whole genome mapping enables identification of transmission events

T. Bosch, E. Verkade, M. van Luit, R. Burggrave, J. Kluytmans, L. Schouls

RIVM, IDS-BSR, Bilthoven

Introduction: Typing of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) using current typing methods yields insufficient discriminatory power to enable identification of transmission events caused by this clade. In this study, we evaluated the capability of a new technique called whole genome mapping (WGM) to type LA-MRSA and elucidate transmission events.

Methods: LA-MRSA and MRSA isolates obtained from outbreaks and non-related isolates LA-MRSA were typed by WGM, spa-typing, MLVA, and PFGE for the validation (n = 70). In addition, isolates cultured from 2 epidemiologically unrelated veterinarians and their family members were used to study possible transmission of LA-MRSA. High molecular weight DNA of >250 kb was transferred into micro-channels in which the molecules were stretched, immobilized, digested with *Afl*II and finally fluorescently stained within the micro-fluidic system. Thereafter the restriction fragments in the micro-channels were photographed and sized in the Argus machine and assembled into ordered, high resolution restriction maps which were imported into the BioNumerics software for further analyses.

Results: Repetitive analysis of the same DNA samples on 4 consecutive days and analysis of DNA obtained from isolates that were sub-cultured for 30 days revealed highly reproducible results with 99% similarity. Three previously well-documented outbreaks were studied. Two outbreaks, LA-MRSA and community acquired MRSA, were confirmed, but WGM revealed major differences between the maps of the third set of hospital acquired MRSA isolates, indicating not all isolates belonged to this outbreak. Analysis of LA-MRSA isolates obtained from 16 unrelated veterinarians yielded only 4 different MLVA-types (MT) and 5 different spa-types and these spa- and MLVA were highly related. Whole genome mapping enabled differentiation in much greater detail and only 2 isolates had indistinguishable WGMs (99.6% similarity). Twenty-four LA-MRSA isolates obtained from 2 epidemiologically unrelated veterinarians and their family members were characterized. Based on Spa-typing and MLVA 21 of the 24 isolates were designated as indistinguishable and all were spa-type t011 and MT398. PFGE grouped the isolates into 2 PFGE clusters each representing one family and similar results were obtained with WGM indicating transmission within the families had occurred. However, in contrast to PFGE, WGM separated the isolates of one of the families into 2 separate groups indicating that colonization within this family had occurred due to transmission with multiple strains.

Conclusion: Until now, PFGE was the best method to differentiate LA-MRSA isolates. However, whole genome mapping is a robust, reproducible and portable technique and seems to be suitable to investigate possible transmission events and outbreaks within the LA-MRSA clade. WGM is considerably cheaper than whole genome sequencing and it will take approximately 3 days from picking up the MRSA colony to comparison of the obtained WGM with other WGMs.

P037

Effect of *Streptococcus pneumoniae* colonization on the upper respiratory tract microbiota in an experimental mouse model

C.L. Krone, G. Biesbroek, T. Zborowski, E.A.M. Sanders, K. Trzcinski, D. Bogaert

University Medical Center Utrecht, Dept. of Pediatric Immunology and Infectious Diseases, Utrecht

Introduction: The bacteria indigenous to the mammalian body are a diverse and important component of a healthy existence. New techniques in sequencing have allowed a greater understanding of the breadth and diversity of these microbial populations while experimental animal models have elucidated mechanisms of commensal-host-pathogen interactions including beneficial immune stimulation and pathogen-resistance. The upper respiratory tract (URT) is a distinct microbial niche of low density bacterial communities and also a portal of entry for many pathogens, including *S. pneumoniae*. Thus far, animal models have been used to study interactions in the URT for a limited number of species at a time. Here we applied a deep sequencing approach to explore, for the first time, the impact of *S. pneumoniae* acquisition on the URT microbiome in a mouse model of pneumococcal colonization.

Methods: Female C57Bl/6 mice aged 3-4 months and 18-23 months were inoculated intranasally with $5x10^6$ CFU *S. pneumoniae* serotype 6B, and at days -1, 3, 7, 14, and 21 post-colonization five mice per group were sacrificed. Post-mortem, nasal lavages and nasal tissue were collected. Pneumococcal CFUs were determined from the culture of nasal lavage and tissue. Bacterial DNA extracted from lavages was subjected to barcoded pyrosequencing of the V5-V7 hypervariable region of the small-subunit ribosomal RNA gene. Sequences were processed using the Mothur pipeline and operational taxonomic units defined on a 97% homology of sequences (OTU).

Results: We observed highly diverse microbial communities in the URT of mice with the overall presence of 15 phyla and approximately 645 OTUs. Profiles were similar within sets of mice (n = 5) at the phyla and family level. However, nave elderly mice showed a different profile than young nave mice, with proteobacteria and actinobacteria dominating in the elderly versus bacteroidetes and firmicutes dominating in the young. The introduction of *S. pneumoniae* into the URT lead to a temporary dominance of pneumococci in the microbiota composition of all mice (OTU and

culture), which was accompanied by a significant decrease in microbiome diversity, as measured by the Shannon and the Simpson indices. Pneumococcal clearance was reached significantly quicker in young compared to elderly mice. With pneumococcal clearance, diversity returned to baseline both in young and elderly mice, and albeit not significant, there was a trend towards a quicker diversification in the young compared to the elderly mice.

Conclusion: We observed differences in URT microbiota composition between young and elderly mice. This was associated with differences in pneumococcal clearance in time. Additional experiments will be conducted to confirm the age-associated differences in respiratory microbiome composition, and their correlation with pneumococcal clearance dynamics. Moreover, our data will be compared to differences in human nasopharyngeal microbiota composition between elderly and young adult individuals to confirm utility of the model.

P038

Second worldwide proficiency study on variable numbers of tandem repeats typing of *Mycobacterium tuberculosis* complex

J.L. de Beer[1], C. Ködmön[2], J. van Ingen[3], P. Supply[4,5,6,7,8], D. van Soolingen[1,3,9]

[1]*National Tuberculosis Reference Laboratory, Laboratory for Infectious Diseases and Perinatal Screening (LIS), Centre for Infectious Disease Control (CIB), National Institute for Public Health and the Environment (RIVM), Bilthoven,* [2]*Surveillance Unit, Tuberculosis Programme, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden,* [3]*Dept. of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen,* [4]*INSERM U1019, Lille, France,* [5]*CNRS UMR 8204, Lille, France,* [6]*Institut Pasteur de Lille, Center for Infection and Immunity of Lille, Lille, France,* [7]*Univ Lille Nord de France, Lille, France,* [8]*GenoScreen, Lille, France,* [9] *Dept. of Pulmonary Diseases, Radboud University Nijmegen Medical Centre, Nijmegen*

Since the introduction of variable numbers of tandem repeat (VNTR) typing as the new standard for typing of *Mycobacterium tuberculosis*, laboratories all over the world have implemented this technique. The most important application of VNTR typing is tracking tuberculosis transmission to facilitate public health interventions, but it also offers the possibility to study the *M. tuberculosis* population structure in different areas of the world.

The quality of VNTR typing of *M. tuberculosis* in worldwide laboratories was investigated for the first time in 2009. The results of 37 laboratories revealed significant problems in the reproducibility of 24-locus VNTR typing, an inter- and intra-laboratory reproducibility of 60 and 72%, respectively. These data spurred an improvement in the laboratory techniques underlying VNTR typing and a higher degree of global standardization of the assay.

In this second proficiency study, with 41 participating laboratories from 36 different countries worldwide, we measured the effects of the technical improvements and higher level of standardization. The overall inter- and intra-laboratory reproducibility increased from 60 to 78% and 72 to 88%, respectively.

Detailed analysis revealed several aspects of VNTR typing that contributed to an improved inter- and intra-laboratory reproducibility, the largest improvement was detected in laboratories that perform VNTR typing by an in-house method and use gel-electrophoresis to detect the size of the PCR products. Ten out of twelve laboratories that performed thisVNTRmethod improved their reproducibility by 45%. This improvement was mainly due to developing skills and experience to perform this manual VNTR method according to a more standardized approach. Furthermore, a significant decline in the number of sample exchange events, number of missing loci and number of systematic errors was observed.

In conclusion, the implementation of an annual worldwide proficiency study resulted in a significant increase in the reproducibility of VNTR typing of *M. tuberculosis*. This will contribute to a more meaningful interpretation of molecular epidemiological and phylogenetic studies. However, global quality control and standardization of VNTR typing remains important and requires a sufficient degree of organization and financial support.

P039

A newly characterized proline-rich surface protein of *Enterococcus faecium* is involved in platelet-binding.

A.M. Guzman Prieto, X. Zhang, F. Paganelli, D. Bierschenk, M. Pape, J. Ouwerkerk, C. Schapendonk, M. Bonten, R. Willems, W. van Schaik

UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Enterococcus faecium has long been considered a commensal of the mammalian gastrointestinal tract. However in the last two decades, *E. faecium* has become an important cause of nosocomial infections that are often difficult to treat due to its increasing resistance to antibiotics. Even though *E. faecium* has recently become an important nosocomial pathogen, little is known about the mechanisms that contribute to colonization and infection of the mammalian host.

As a first step towards the identification of genes involved in these processes we performed a transcriptome analysis of *E. faecium* E1162, recovered from a bloodstream infection, during mid-exponential growth at 25 °C and 37 °C. Differences in gene expression between 25 °C and 37 °C were relatively limited, perhaps reflecting the homeostatic nature of enterococcal physiology under permissive growth conditions. Thirty-three genes showed significantly higher expression at 37 °C than at 25 °C. One of the most highly upregulated (4.3-fold) genes during growth at 37 °C is predicted to encode a 48-kDa surface protein (locus-tag: EfmE1162_0376) with a LPxTG-type anchor. The C-terminal part of the protein contains three proline, threonine and glutamic acid-rich repeat regions, while the N-terminal domain has no orthologs in bacteria other than *E. faecium*. Due to the number of proline residues present, we called this protein PrpA for proline-rich protein A.

Full length PrpA and separately its N- and C-terminal domains were overexpressed with N-terminal His_6-tags in *E. coli* and purified. Polyclonal antibodies against the full-length protein were raised in rabbits. The antibodies were used to determine the relative levels of PrpA in exponential and stationary phase cultures of *E. faecium* E1162 by flow cytometry. This showed that PrpA is present at highest levels in exponentially growing cells at 37 °C, while the surface exposed levels were lower in stationary phase or when grown at 25 °C. The localization of PrpA during exponential phase was assessed using confocal microscopy and electron microscopy. PrpA was found to be distributed exclusively towards the poles of the bacteria. Polar localization has been described in other gram-positive bacteria, for instance to concentrate the adhesion machinery facilitating host invasion.

The purified N-terminal domain of PrpA but not the C-terminal domain was able to bind the blood plasma proteins fibrinogen and fibronectin. This observation led us to further investigate the potential role of PrpA in the interactions of *E. faecium* with platelets, as these interactions have recently been shown in other bacteria to contribute to pathogenesis. Using flow cytometry, we showed that both full length PrpA and its N-terminal domain interact with activated and inactivated platelets, possibly mediated through fibrinogen. Next, we constructed a double cross over markerless mutant (*fprpA*) and an *in-trans* complemented strain in *E. faecium* E1162. We showed by confocal microscopy that the prpA deletion mutant does not aggregate platelets to the same level as the wild type strain E1162. We have also found antibodies against PrpA in patients with bacteremia. Together our data indicate that PrpA may contribute to pathogensis of *E. faecium*-mediated infections of hospitalized patients.

P040

Population structure and dynamics of ESBL producing *Escherichia coli* in a Dutch hospital

A.A. Wattel[1], S.A. Boers[2], D.M.C. de Jongh[1], S. Gutierrez[1], R. Jansen[2], J.P. Hays[1], W. Goessens[1]

[1]*ErasmusMC, Medical Microbiolgy, Rotterdam,* [2]*Regional Laboratory of Public Health, Molecular Biology, Haarlem*

Introduction: Since the emergence of resistance genes an increasing amount of bacteria is subject of studies for genetic relationships. Among these bacteria, *Escherichia coli* plays an important role as this bacterium is involved in many infections in hospitals. Therefore we investigated the population structure and dynamics of *E.coli,* carrying resistance genes, in 2008 and 2010 in Rotterdam, the Netherlands.

Materials and methods: All *E.coli* strains in ErasmusMC, Rotterdam, in 2008 and 2010 were screened on ESBL phenotype using CLSI criteria. Only isolates that were confirmed as ESBL producers or when confirmation was doubtful were included. From a total of 5734 unique *E.coli* isolates, i.e. one *E.coli* strain a patient, 335 isolates met the criteria. We selected 213 strains for further analysis by PCR on CTX-M, OXA, TEM, SHV and plasmid mediated AmpC genes. These isolates were also genotyped using High throughput Multilocus sequence typing (HiMLST).

Results: ESBL producing *E.coli* increased significantly from 133/2800 (4.8%) in 2008 to 202/2931 in 2010 (6.9%). Further, pAmpC containing isolates also increased significantly from 0/2800 in 2008 to 13/2931 (0.4%) in 2010. The Dutch ESBL *E.coli* genotypes were representative of current *E.coli* genotypes circulating in Europe. The international clone, ST 131, was present in our hospital too (64 of 194 strains). This clone harbored different combinations of ESBL genes. Although nine strains of the ST 131 did not contain CTX-M genes, this gene was significantly present, p = 0,0008.

Discussion and conclusion: In our hospital ST 131 contained different combinations of ESBL genes, but CTX-M was significantly involved. The *E.coli* population structure in Rotterdam is similar to that observed in European countries. This suggests that ESBL and pAmpC positive *E.coli* isolates at ErasmusMC are introduced into the hospital from the community rather than being associated with the expansion of a single genotypic clone. Further, the increase in ESBL phenotype among *E.coli* isolates provides further evidence for the successful transmission of ESBL-gene containing plasmids between different *E.coli* genotypes. Following the flow of resistance genes has become essential nowadays, therefore HiMLST is a good technique to use for this purpose.

P041

Phenotypical testing for carbapenemase activity

W.K. van der Zwaluw, A. Haan, M.E.O.C. Heck, H. Bijlmer

RIVM, CIb, Bilthoven

Introduction: The emergence and spread of carbapenemase producing gram-negative rods is a worldwide emerging public health threat. While most research is focused on the detection of genetic markers for resistance mechanisms,

phenotypic tests have mostly remained unchanged. However, where PCR methods can only detect the targets for which they are designed, phenotypical testing will detect strains with that characteristic, irrespective of the underlying genetic mechanism. Recently, a new test was published to detect the capability of an isolate to hydrolyze carbapenems, the Carba NP test. Up until now, the ability to hydrolyze carbapenems was tested using the Modified Hodge Test. However, interpretation of this test is highly subjective and both specificity and sensitivity leaves much to be desired. The Carba NP test claims to be 100% specific and sensitive and to be faster.

Methods: The Carba NP test relies on the creation of an enzymatic bacterial suspension from each isolate. This suspension is then added to mixture of imipenem monohydrate and phenol red. Upon hydrolysis of the imipenem by carbapenemases within the enzymatic bacterial suspension, pH levels will decline and the phenol red solution will turn from reddish purple to orange / yellow. Forty isolates of various species of *Enterobacteriaceae* and non-fermentors were tested with this method, both with and without known carbapenemase genes, with both low and high meropenem MIC's and also including reference strains.

Results: The Carba NP test was positive for isolates containing a carbapenemase gene and negative for those lacking these genes, regardless of their MIC's. One false-positive isolate was found to be carrying a carbapenemase gene not routinely tested for. One false-negative isolate is believed to carry a non-functional NDM gene, which shall be further investigated.

Conclusion: The phenotypic approach to detect the ability of isolates to hydrolyse carbapenems was already successful in identifying an additional carbapenemase positive isolate. The Carba NP test offers a fast (< 3 h) and reliable method to do so. However, this method requires a large amount of imipenem (0.3 mg) for each isolate, making it a costly test (9 euro/isolate) for general screening purposes. Efforts to modify the test to reduce cost are being made at this time.

P042

Characterization of extended spectrum bèta-lactamase-producing, multidrug-resistant *Enterobacteriaceae* in culinary herbs imported from South-East Asia

K.T. Veldman

Central Veterinary Institute of Wageningen (UR), Bacteriology & TSEs, Lelystad

Objectives: As part of a national study on the occurrence of ESBL-producing *Enterobacteriaceae* in food samples, imported herbs from different South-East Asian countries were included.

Methods: The Netherlands Food and Consumer Product Safety Authority (NVWA) in Wageningen collected 32 fresh herb samples imported from South-East Asia. Ten gram of each sample was selectively enriched in 90 ml Luria Bertani broth with 1 mg/L cefotaxime. After 16 - 20h incubation at 37 °C all samples were inoculated on MacConkey with 1 mg/L cefotaxime as well as on Brilliance ESBL agar. After 16 - 20h incubation at 37 °C isolates with growth typical for *Enterobacteriaceae* were pure cultured on blood agar plates and sent to the Central Veterinary Institute (CVI) in Lelystad for further analysis. The analysis included susceptibility testing by broth microdilution according to ISO 20776-1:2006, screening for resistance genes with micro-array (AMR-ve 05, Alere Technologies), PCR and sequencing of specific bèta-lactamase and plasmid mediated quinolone resistance (PMQR) genes, transformation of plasmids in DH10B cells followed by PCR bases replicon typing (PBRT) of plasmids. To determine the location of the ESBL and/or PMQR genes S1-PFGE was performed followed by Southern hybridization. Finally, the bacterial isolates were identified to species level by MALDI-TOF mass spectrometry at the GD Animal Health Service in Deventer.

Results: The study resulted in 24 cefotaxime resistant *Enterobacteriaceae* obtained in 32 imported herb samples, which comprised of 18 samples from Thailand (Morning Glory, Acacia and Betel Leaf), 9 samples from Vietnam (Parsley, Rau Ma, Houttunya leaf and Peppermint) and 5 samples from Malaysia (Holy Basil and Parsley). Array analysis revealed 17 isolates (70.8%) with ESBL genes, 2 isolates (12.5%) with mutations in the ampC promotor region, 2 isolates (8.3%) with solely none-ESBL bèta-lactamase genes and 3 isolates (= 12.5%) in which no bèta-lactam genes could be detected. The latter 3 isolates were excluded from this study. The remaining 21 isolates were identified as *Klebsiella pneumonia* (n = 9), *Escherichia coli* (n = 6), *Enterobacter cloacae* (n = 5) and *Enterobacter* spp. (n = 1). All isolates tested were remarkably multidrug-resistant showing resistance against 4 to 10 different antibiotic classes. Variants of CTX-M enzymes (CTX-M-9,–14,–15 and -40) were predominantly found (n = 13) followed by SHV-12 (n = 4). Furthermore, a high number of isolates with PMQR genes were detected (n = 18). PCR and sequencing revealed 2 isolates with qnrB, 4 isolates with both aac(6')-1b-cr and qnrB, 2 isolates with both qnrB and qnrS and 10 isolates with qnrS. In almost all cases the ESBL and quinolone resistance genes were located on the same plasmid. These plasmids were replicon typed as IncF, IncR, IncHI1 or IncHI2. In 7 isolates the ESBL genes were located on a none-typeable plasmid. Finally, in one *E. coli* isolate hybridization experiments revealed both $bla_{CTX-M-15}$ and qnrS were chromosomally located.

Conclusion: Imported culinary herbs from South-East Asia are a potential source for contamination of food with multidrug-resistant bacteria. These bacteria harbour genes against several different antibiotic classes, including ESBL's and plasmid mediated quinolone resistance genes.

P043

Mechanism of inactivation of *Pseudomonas aeruginosa* by cold gas plasma

B.K.H.L. Boekema[1], C.A.J. Van Gils[2], S. Hofmann[2], P.J. Bruggeman[2], E. Middelkoop[3], G.M.W. Kroesen[2]

[1]VSBN, Preklinisch onderzoek, Beverwijk, [2]Eindhoven University of Technology, Eindhoven, [3]VU University Medical Center, Plastic, Reconstructive and Hand Surgery, Amsterdam

Introduction: Bacterial contamination is a constant threat in burn wound care and needs to be treated. It is however important to keep a balance between inactivating the bacteria and maintaining the wound healing potential. Cold atmospheric pressure plasmas might provide additional means to reduce the bacterial load in a burn wound. Atmospheric pressure plasmas have been used for many years for different applications. Atmospheric pressure plasmas deliver electrons, ions, UV radiation and an electric field, which together are effective in killing bacteria. We studied the effects and mechanism of cold argon plasma treatment on in vitro inactivation of *Pseudomonas aeruginosa*, which is commonly isolated from burn wounds.

Methods: For the treatment, a pulsed cold atmospheric plasma jet (13.56 MHz micro-jet) was used. Bacteria were treated in physiological salt with plasma at different settings for different times. Surviving bacteria were counted by plating dilutions.

Because reactive radicals in plasma can interfere with the healing process, cell cultures of keratinocytes or fibroblasts in physiological salt were treated with cold plasma after which culture medium was added. To estimate cell damage due to the treatment, activity of the cells was quantified with a tetrazolium based assay.

To elucidate the mechanism of plasma mediated inactivation, we studied gas temperature, absolute UV irradiance, emitted ions (mass spectrometry) and induced liquid chemistry (liquid ion chromatography and hydrogen peroxide concentration).

Results: Bacterial inactivation reached up to 7-log reduction after 1 min. Plasma treatment leads to a pH decrease in non-buffered solutions which is critical for plasma activity. Inactivation was hardly observed in phosphate buffered saline or culture broth. The extent of bacterial inactivation was further related to the distance, duty cycle and treatment time. Plasmas operated at low duty cycles (10-20%) reduced the viability of fibroblasts. Higher settings did not damage fibroblasts. Plasma treatments of keratinocytes did not result in loss of activity.

The effects of heat, ion- and UV-fluxes, electric fields, and gas flow were estimated not to have a prominent direct effect on bacterial inactivation. Rather, plasma exerts its effect through liquid phase chemistry, most likely via HNO_2, $ONOO^-$ and H_2O_2.

Conclusion: Non-thermal argon plasma can be used to kill bacteria and yet preserve the viability of skin cells. The bactericidal effect can be ascribed to plasma induced liquid chemistry, leading to the production of stable and transient chemical species.

P044

Detection of *Streptococcus pneumoniae* in saliva samples from Dutch primary school children

A.L. Wyllie, M.L. Chu, M.H.B. Schellens, D. Bogaert, E.A.M. Sanders, K. Trzcinski

Wilhelmina's Children Hospital, UMC Utrecht, Dept. of Pediatric Immunology and Infectious Diseases, Utrecht

With the introduction of pneumococcal conjugate vaccines, more in-depth carriage studies are required to monitor the effects of vaccination. Here, we investigated saliva as a means of detecting *Streptococcus pneumoniae* colonization for potential use in surveillance on pneumococcal carriage. We were inspired by Gundel and Okura (Zentr.Hyg., 1933) describing rates of up to 80% pneumococcal carriage in healthy schoolchildren, detected in saliva using a mouse inoculation method. We applied conventional culture and molecular methods to detect pneumococci in uncultured (raw) and culture-enriched saliva collected from school-children, with the aim to compare the sensitivity of various diagnostic methods.

Samples were collected from 49 students (age 5 to 10 years, median 8) of a rural school near Utrecht, on a single morning in June 2012. Each child spat into a disposable container. Samples were placed on wet ice and transported to the lab within 2 hours. Blood agar plates supplemented with 5 mg/l gentamicin were inoculated with 100 ul of sample and the remaining raw saliva was frozen at -80 °C. After overnight incubation at 37 °C and 5% CO_2, cultures were inspected for the presence of *S. pneumoniae*. Next, all bacterial growth was harvested in 1 ml of 10% glycerol in BHI and stored at -80 °C. DNA was extracted from 100 ul of thawed raw and culture-enriched samples, using either a modified Qiagen DNeasy kit protocol or bead-beating of the samples followed by magnetic separation of DNA with Agowa reagents. Purified DNA was tested by quantitative-PCR (qPCR), targeting *S. pneumoniae* specific genes lytA and piaA. Samples were considered positive with CT values for both genes < 40.

Saliva cultures show abundant polymicrobial growth, which allowed detection of *S. pneumoniae* by eye in 2 children only (4%). To compare efficiency of DNA isolation methods, DNA templates from raw and culture-enriched samples from 15 randomly selected children were tested by qPCR. The lytA-specific signal was stronger for templates purified with Agowa compared to Qiagen protocol (average D1.24 CT for raw and D6.94 CT for culture-enriched samples). Thus, DNA was isolated from all samples using the Agowa protocol.

Altogether 41 (84%) children were identified as qPCR-positive for *S. pneumoniae* in culture-enriched samples compared to 30 (61%) in raw saliva samples. Culture-enrichment increased the strength of lytA-specific signal (average D6.37 CT) compared to raw samples. All children qPCR-positive in raw saliva were also positive in culture-enriched samples. There was a negative correlation between age and the quantity of lytA detected in qPCR in raw samples (Spearman's r = 0.3, p = 0.03).
Isolation of live *S. pneumoniae* from saliva by conventional culture is extremely difficult due to its polymicrobial nature. Conventional culture limitations were addressed by applying a combination of culture-enrichment and sensitive molecular methods. This resulted in the detection of high rates of pneumococcal carriage in schoolchildren that matched rates of the pre-antibiotic era. The simplicity of sample collection and the high sensitivity of pneumococcal detection suggest that saliva could be considered as an alternative to nasopharyngeal swab sampling in surveillance on pneumococcal carriage in children.

P045

Detection of *Streptococcus pneumoniae* in dried saliva spots: exploratory study on alternative diagnostic approaches in surveys on pneumococcal carriage

C.L. Krone, K. van de Groep, E.A.M. Sanders, D. Bogaert, K. Trzcinski
University Medical Center Utrecht, Pediatric Immunology and Infectious Diseases, Utrecht

Saliva is an easily accessible body fluid and as such there is growing interest in exploring it for a range of diagnostic purposes. Historically, saliva was the first specimen tested in surveillance studies on pneumococcal carriage. Combined with a sensitive mouse inoculation method, it resulted in colonization rates in healthy adults in the pre-antibiotic era between 45% and 60%. However, in order to use saliva for pneumococcal carriage surveillance the sample should be transported at low temperatures, preferably snap frozen. These requirements make saliva unattractive for large surveillance studies. Interestingly, Welsh and Camilli recently reported (MBio, 2011) on the relative tolerance to desiccation of *S. pneumoniae*. The aim of this study was to determine the feasibility of using dried saliva spots (DSS) as an alternative to raw saliva collected on dry ice for pneumococcal carriage studies.
Saliva was collected from healthy adult volunteers and spiked with 10^6 CFU/ml of *Streptococcus pneumoniae* clinical strains of serotype 1, 2, 3, 4, 6B, 11A, 19A, or 19F; 100 ul was applied to Whatman 903 Protein Saver cards (cotton fibers), and allowed to air-dry for 2 hours in ambient conditions. DSS were stored sealed in plastic bags with a desiccant pack at 30 °C, room temperature (RT), 4 °C, or -80 °C for up to 35 days. DNA was isolated from spots with a modified DNeasy Blood and Tissue kit (Qiagen), and tested for pneumococcal presence in quantitative-PCRs (qPCR) targeting the autolysin (LytA) and the ABC transporter (PiaA) genes.
Dried saliva spots processed immediately after drying showed equal qPCR pneumococcal specific Ct values as freshly spiked raw saliva samples. Serial dilution of pneumococci showed that the limit of detection of DSS was 10^4 CFUs per spot, an equivalent of approximately 250 CFUs per 1.25 ul of raw saliva sample, corresponding to the volume of DNA tested per sample in qPCR. Pneumococcal DNA was stable for up to 10 days in DSS stored at temperatures <RT and for up to 7 days in DSS stored at 30 °C. After these time-points CT values became highly variable, but presence of pneumococcal DNA was still detected in DSS stored up to 35 days at any temperature tested. There were no differences between results for various clinical strains tested in the study.
We conclude that pneumococcal DNA is stable in DSS stored with desiccant for up to one week in a wide range of temperatures. DSS may be considered as an attractive alternative to nasopharyngeal swab samples in surveillance studies on pneumococcal carriage. Since collection of saliva does not require trained personnel and DSS allow robust transport conditions, the method could be particularly useful in studies conducted in remote settings.

P046

A MLST for fastidious growing *Brachyspira* species directly from clinical samples: targeting the 'dead, scattered, buried, and hidden in the crowd'-faction

L.J. Westerman[1], H.V. Stel[2], M.E.I. Schipper[1], D.S.A. Ahad[1], M.J.M. Bonten[1], J.A. Wagenaar[3], J.G. Kusters[1]
[1]*University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*Tergooiziekenhuizen, Dept. of Pathology, Hilversum,* [3]*Faculty of Veterinary Medicine, Utrecht University, Dept. of Infectious Diseases and Immunology, Utrecht*

Introduction: *Brachyspira* species are fastidious, obligate anaerobic, gram-negative bacteria that inhabit the large intestine of various animals and humans. Several species are established pathogens whereas others are considered commensals. Two species are known to cause human intestinal spirochaetosis: *Brachyspira pilosicoli* and *Brachyspira aalborgi*. *B. aalborgi* is notoriously difficult to culture, with only a few successful attempts described in literature. Next to these two well-known human species, there is also molecular evidence for a third species, with the proposed name *'Brachyspira hominis'*. To date, *'B. hominis'* has never been cultured and it has been hypothesised that it represents a true' unculturable. The scarcity of *B. aalborgi* and the absence of *'B. hominis'* isolates precludes solid phylogenetic studies.
Aim of Study: To design a MLST for *Brachyspira* species directly from clinical samples.
Methods: Nine criteria were established that would have to be met for a successful MLST:
A) Basic criteria: Housekeeping genes should be targeted. Only one copy of these genes should be present in all four whole genome sequences currently available (*B. pilosicoli*, *B. hyodysenteriae*, *B. murdochii* and *B. intermedia*). Those sequences should differ in nucleotide composition.
B) *In silico* criteria: These sequences should be very different from other living organisms using BLAST.
An amplicon specific for *Brachyspira* species of less than 400 bp should be targeted.
The designed primers should not amplify any other organism in primer-BLAST.
C) *In vitro* criteria: The primers should not amplify known *Brachyspira*-negative samples in vitro (isolates, biopsies and faecal samples).
The primers should amplify known *Brachyspira*-positive samples (type-strains, isolates, biopsies and faecal samples). These products should be sequence-able.
Brachyspira-positive biopsy- and faecal-samples were taken from a previous study and several clinical isolates from the veterinary faculty were used.
Alignment of sequences and protein translations were made using Seaview. Phylogenetic analysis was performed in Splitstree version 4.12.6, built 24 May 2012.
Results: 87 genes fulfilled the *in silico* criteria, 30 of these were tested against the *in vitro* criteria. Seven primer-sets were designed that fulfilled all nine criteria: the first part of the 16S rDNA-gene, the NADH-oxidase gene, DNA polymerase III bèta clamp subunit, DNA-directed RNA polymerase alpha subunit, ATP synthase gamma unit, rod shape-determining protein and GTP-binding protein. Eight trees based on nucleotide-sequences were generated: one for each locus and one concatenated tree of all samples and six protein trees. Samples clustered consistently in two branches: a *B. aalborgi*-branch and a *B. pilosicoli*-branch. *'B. hominis'* samples did not cluster consistently together, but mostly clustered with *B. aalborgi*. Several samples from both species did not cluster in their 16S-identified branch, but in the other branch.
Conclusion: Using this approach, we successfully designed a MLST targeting housekeeping genes directly in clinical samples. Up to now 60 (faecal and biopsy) samples have been successfully analysed.
The obtained data strongly suggest that:
1. *'B. hominis'* is not a separate species, but a 16S-variant of *B. aalborgi*.
2. There is evidence for horizontal gene transfer between *Brachyspira* species.

P047

Successful production, secretion and isolation of *Staphylococcus* antigens using *lactococcus lactis* as a host

J. Neef[1], G. Buist[1], D.G.A.M. Koedijk[1], T. Bosma[2], F. Milder[3], J.M. van Dijl[1]
[1]*University Medical Center Groningen, Dept. of Medical Microbiology, Groningen,* [2]*BioMaDe Technology Foundation, Groningen,* [3]*University Medical Center Utrecht, Medical Microbiology, Utrecht*

Introduction: For the development and testing of novel proteinaceous antigens of pathogenic bacteria the isolation of these targets is needed. Since the cytoplasmic production of many secreted proteins of *Staphylococcus aureus* with conventional *Escherichia coli* or *Lactococcus lactis* expression systems resulted in low yields, a new approach was followed for their production.
Methods: In this approach, the His-tagged proteins were secreted by *L. lactis* after induced expression using a natural signal peptide for Sec-dependent export from *L. lactis*. A set of expression vectors for protein production in *E. coli* has been used to construct derivative vectors for the expression of N- or C-terminally His-tagged fusion proteins with or without a TEV-cleavage site for the removal of the His-tag after protein isolation. In addition to the His-tag, an Avi-tag has been cloned into these vectors for the biotinylation of isolated proteins.
Results: The tagged proteins were isolated directly from the culture medium using metal affinity chromatography. Lysis of *L. lactis* cells, resulting in a release of cytoplasmic proteins, and degradation of the expressed fusion proteins were severely reduced by using a lactococcal strain that is mutated in the major autolysin AcmA and the membrane-associated protease HtrA. Notably, some of the selected cell surface-exposed *S. aureus* proteins also remained cell wall-attached upon production in *L. lactis*. These proteins could, however, be released from the *L. lactis* cells by treatment with 6M urea or 2M KSCN after which their purification was possible. After isolation the proteins had or could retain their natural activity. More than twenty-five staphylococcal antigens, which are naturally secreted, or membrane- or cell wall-bound (covalently or non-covalently), have been successfully produced and/or isolated. For one of the proteins, besides a His-tag, also an N-terminal AVI-tag was added for site-specific protein labeling by biotinylation. Cytoplasmic expression of this fusion in *E. coli* resulted in the formation of inclusion bodies while secretion from *L. lactis* resulted in a soluble protein which, after isolation, could be enzymatically biotinylated.
Conclusion: We believe that this approach will be beneficial for successful overexpression, isolation and labeling of functionally active secreted proteins of gram-positive

bacteria that can among others be used for the testing of novel proteinaceous antigens.

P048

Antibiotic disks stable for many months; do not throw them away

W.C. van der Zwet, C.D. Viceisza-Blijden, C.J. Passchier
Deventer Ziekenhuis, Laboratorium voor Medische Microbiologie en Infectiepreventie, Deventer

Introduction: Although susceptibility testing is more and more automated, disk diffusion remains a standard procedure. The manufacturer of antibiotic disks used in our laboratory (Oxoid), advises to discard working supplies of disks after a maximum of 1 week after opening. This results in financial losses. Our hypothesis was that this worldwide discarding advise is not applicable for Dutch microbiological laboratories with secured temperate control and therefore we could save expenses. This study was performed to investigate the stability of antibiotic disks for over a period of 9 months.
Methods: From September 2011 to July 2012, every month an investigational cartridge from several antibiotics were opened and afterwards stored as the antibiotic working supplies that were used in every day practice, until moment of testing. During the study, cartridges of the same lotnumber were used. Disk diffusion tests were performed in December 2011 and July 2012. Before these tests, cartridges were also weekly opened and stored in the preceding month. For disk diffusion testing, *S.aureus* (ATTC strain 25922) and *E.coli* (ATCC strain 29213) were used. All testing was performed by one microbiology technician following EUCAST procedures. Zone diameters were measured and an assesment was made whether zones were outside accuracy control limits. Furthermore, in August 2012, 4 other microbiology technicians performed disk diffusion on the cartridges of January 2012, to investigate individual differences in results.
Results: In December 2011, 94 days after starting the study, for both *E.coli* and *S.aureus* disk diffusion zones were within EUCAST quality control limits. The maximum loss of zonediameter was 2 mm (min-max: 0-2). In July 2012, 307 days after starting the study, 5 antibiotics showed a loss of zonediameter of > 3 mm (min-max: 0-5, *S.aureus*: ampicillin, and *E.coli*; ampicillin, meropenem, ceftazidime and nalidixic acid). Three antibiotics showed zonediameters below the quality control limit (*S.aureus*: ampicillin, *E.coli*: ampicillin, augmentin and ceftazidim). The comparison in between technicians showed differences between smallest and largest measured zones varying from 1 to 5 mm.
Conclusion: Disk diffusion zones are stable for periods longer than 7 days. It depends on the ATCC strain if zonediameters fall below the quality control limit sooner or later. Variation in reading disk diffusion zones is subject to technician to technician variability. If a zonediameter loss of 2 mm is considered acceptable, in our laboratory disks can be used for 150 days. Further studies, with the use of multiple lotnumbers by several technicians, are necessary to establish the definitive tenability of antibiotic disks.

P049

Why using LC- MS/MS in microbiological diagnostics?

H. Trip, J.A. Majchrzykiewicz, A. Paauw
TNO, CBRN Protection, Rijswijk

Fast detection and identification of pathogenic bacteria is highly important for an adequate and fast response to biological warfare agents as well as in combating infections in clinical environments. The use of mass spectroscopy in identification of micro-organisms has made great progress in the last two decades and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy is now employed in many hospitals as a fast and reliable alternative to traditional identification methods. However, identification with the standardized and commercialized MALDI-TOF based methods is usually limited to the species-level and does not provide information on characteristics like antibiotic resistance and virulence factors. The present work describes a study of the applicability of a liquid chromatography-tandem MS (LC-MS/MS) based method for identification of bacteria, including a generic, simple and fast sample preparation procedure. LC-MS/MS analysis after trypsin digestion of whole cell extracts allows for sequencing of large numbers peptides in one sample, and therefore identification on the sub-species level. Six gram-negative and six gram-positive bacteria were tested with the same sample preparation procedure, consisting of resuspending colony material, a 10 minutes heating step and one hour of trypsin digestion. Filtered samples were analyzed by LC-MS/MS, and proteins were identified based on peptide mass fingerprinting using the Mascot search algorithm for protein identification. The sample preparation method worked sufficiently for the gram-negative bacteria, resulting in the identification of 50 to 70 proteins (with two or more peptide sequences identified) per sample. For the gram-positive bacteria, significantly less proteins were identified (ranging from 5 to 50 per strain), likely due to inefficient cell lysis and therefore lower yield of tryptic peptides. Work is in progress to improve the tryptic peptide yield, while remaining the method generic, simple and fast. Nevertheless, even in case of the sample with the lowest number of identified peptides, the species could be determined. Not surprisingly, peptides of ribosomal proteins were identified in all tested strains, but also transcription elongation factor, GroEL, the a and b subunit of F-ATPase, phosphopyruvate hydratase and others were overrepresented.
LC-MS/MS has the capability to identify micro-organisms to the strain level and it has the potential to screen simultaneously for important biomarkers (e.g. ESBL, VanA, MecA, PVL). Conclusively, most likely the next wave in the improvement of microbiological identification will be LC-MS/MS.

P050

Technical evaluation of SpectraCellRA as a bacterial typing tool for *Pseudomonas aeruginosa* strains.

H.F.M. Willemse, T. Bakker Schut, G. Puppels, W. Goessens
Erasmus MC, Dept. of Medical Microbiology, Rotterdam

Introduction: Over the past decades, a significant increase of healthcare associated infections (HAI) caused by gram-negative bacteria has been observed. Acquired antimicrobial resistance in these bacteria is probably the most anticipated problem in hospitals.
Effective prevention of HAI requires bacterial typing, since this is a powerful tool for the identification of transmission pathways. But not for all pathogens a reliable typing method is available that can be used in daily routine. Therefore, we have developed a protocol for the typing of *Pseudomonas aeruginosa* strains using the SpectraCellRA typing system. In this study the technical evaluation using well-defined strain collections is described.
Methods: Three well documented collections (n = 150) were used to obtain proof of principle for the use of the SpectraCellRA system as a bacterial typing tool.
Isolates were cultured on Trypticase Soy agar plates, biomass was suspended in sterile distilled water, transferred onto a MicroSlide and allowed to dry. Spectroscopic fingerprints were obtained using a SpectraCellRA analyzer (RiverD international BV). Similarities between isolates were calculated from the squared Pearson correlation coefficients and displayed in a 2 dimensional (2D) checkerboard plot.
Results: Analyzing a set of 50 isolates in triplicate resulted in a repeatability of 96%. The discriminatory power obtained matched that of established typing methods such as PFGE and MLVA. In the two clinical sets used, previously described outbreak specific clusters could be discriminated from non-related isolates.
Conclusion: Reliable and rapid bacterial typing allows infection control professionals to act in a timely manner. Due to the concordance to high resolution genetic typing methods, the ease of use and repeatability, the SpectraCellRA system is a useful typing tool for *P. aeruginosa* strains.

P051

Determination of the complete genome sequences of 20 isolates of *Mycoplasma pneumoniae*

C. Vink, E.B.M. Spuesens, R.W.W. Brouwer, T. Hoogenboezem, C.E.M. Kockx, W.F.J. van IJcken, A.M.C. van Rossum
Erasmus MC, Laboratory of Pediatrics, Rotterdam

Introduction: *Mycoplasma pneumoniae* is a significant cause of pneumonia and other respiratory tract infections. Despite the relatively small genome size of this bacterium (~816 kilobasepairs (kb)), only three full genome sequences are currently available from sequence databases. As a consequence, little is known on the genetic variability of *M. pneumoniae* isolates and the relationship between genotype and virulence of (clinical) isolates. We therefore set out to determine the genome sequence of 20 *M. pneumoniae* isolates obtained from both symptomatic and asymptomatic human subjects.
Methods: Whole-genome sequencing of the 20 *M. pneumoniae* isolates was performed on an Illumina HiSeq 2000 sequencer using the paired-end 100 bp sequencing protocol. Between 0.9 and 2.5 gigabases (gb) of DNA sequence was generated for each of the isolates. Thus, a 1224- to 3300-fold genome coverage was obtained. The generated sequences were *de novo* assembled using Abyss (version 1.3.4). The assembled scaffold sequences were compared to the known genome sequence of a reference *M. pneumoniae* strain (M129), using MUMmer (version 3.23).
Results: For each of the 20 isolates, genome assemblies were generated that ranged between 772 and 829 kb in length. As opposed to reference strain M129, four isolates with significant rearrangements were found. In one these isolates (I12-1149-08), a large translocation was found, such that a ~62 kb sequence appeared to have been transferred from position 350-410 kb in the reference genome to position 190-250 kb in the I12-1149-08 genome. In isolate I12-1149-10, a sequence of ~30 kb at position 600-630 kb was found to be duplicated. Finally, in isolates I12-1149-10, I12-1149-13 and I12-1149-14, a ~6-kb sequence was inserted at position ~700 kb. In addition to these relatively large rearrangements, we also identified numerous deletions and insertions in the genome sequences in comparison with the M129 genome. In general, this sequence variation was found to be shared between subsets of isolates and is not randomly distributed over the genome.
Conclusion: Although unique and relatively large rearrangements were identified in the genomes of some *M. pneumoniae* isolates, our data indicate that *M. pneumoniae* is a genetically stable species. Combined with clinical data, a more detailed analysis of the sequencing information should reveal a putative association between *M. pneumoniae* genotype and virulence.

P052

Immunogenicity of 13-Valent pneumococcal conjugate vaccine administered according to 4 different primary immunisation schedules in healthy infants: the PIM study

G.A.M. Berbers[1], J. Spijkerman[2], M.J. Knol[1], R.H. Veenhoven[3], K.E.M. Elberse[1], P.G.M. van Gageldonk[1], A.J. Wijmenga-Monsuur[1], H.E. de Melker[1], E.A.M. Sanders[2], L.M. Schouls[1]

[1]RIVM, CIB, Bilthoven, [2]UMC Utrecht, Dept. of Paediatric Immunology and Infectious Diseases, Utrecht, [3]Research Centre Linnaes Institute, Spaarne Hospital, Hoofddorp

Introduction: Immunization schedules with pneumococcal conjugate vaccine (PCV) differ largely between countries with respect to number of doses, interval between doses, and age at first dose. To assess the optimal primary schedule in the first 6 months of life, we performed an open-label parallel-group randomized controlled trial in the Netherlands to compare immunogenicity of 13-valent PCV in four different immunization schedules.

Methods: We randomly assigned 400 healthy at term' born infants to receive PCV13 at: 2-4-6 months, 3-5 months, 2-3-4 months or 2-4 months in the primary series with a booster dose at 11 months. All infants received DTaP-IPV-Hib vaccine at2-3-4and 11 months. Blood samples were collected 1 month after the primary series, at 8 and 11 months, and 1 month after the booster dose. Primary outcome measure was IgG antibodies against PCV-13-included serotypes at 12 months of age measured by multiplex immunoassay.

Results: After the booster, virtually no differences in GMC levels were observed and seroprotection levels (> 035 g/ml) were similar for all schedules (94%-100%). After the primary series, the 2-4-6 schedule was found superior to the 3-5, 2-3-4 and 2-4 schedules for 3, 9 and 10 serotypes, respectively. Likewise, the 3-5 schedule was superior to the 2-3-4 and 2-4 schedules for 5 and 11 serotypes, respectively. The 2-3-4schedule was superior to the 2-4 schedule for 5 serotypes. Post-primary seroprotection rates ranged between 76%-100%, but were lower for serotypes 6B or 23F

Conclusion: Optimal timing proved to be more important than number of doses preferring the 2-4-6 and the 3-5 over the 2-3-4 and 2-4 schedules. However, the optimal primary schedule based on effectiveness of the immune responses has to be weighed against the need for early protection depending on country-specific pneumococcal epidemiology, especially in the absence of established herd protection.

P053

Mycoplasma pneumoniae and *Mycop*lasma genitalium encode DNA repair proteins that specifically cleave abasic DNA sites and possess divalent cation-dependent 3'>5' exonuclease activity

C. Vink, P.E. van der Spek, T. Hoogenboezem, A.M.C. van Rossum

Erasmus MC, Laboratory of Pediatrics, Rotterdam

Introduction: The DNA recombination and repair machineries of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* are predicted to be composed of a limited set of approximately 11 proteins. The function of one of these predicted proteins was inferred from its homology with proteins that belong to the so-called Endonuclease IV (Endo IV) enzyme family. These enzymes may play a role in the recognition and repair of apyrimidinic/apurinic (AP or abasic) sites in DNA. As such an activity may be crucial in the bacterial life cycle, we set out to study the activities of the Endo IV-like proteins (termed NfoMpn and NfoMge, respectively) encoded by *M. pneumoniae* and *M. genitalium*.

Methods: The proteins encoded by open reading frame (ORF) MPN328 of M. pneumoniae (NfoMpn) and ORF MG235 of *M. genitalium* (NfoMge) were expressed in *Escherichia coli*, purified, and studied for their ability to interact with normal' DNA substrates and substrates containing abasic sites.

Results: The orthologous proteins NfoMpn and NfoMge were both found to be inactive on normal DNA substrates in the absence of divalent cations in the reaction mixture. However, under the same conditions, both proteins exhibited extensive, specific cleavage of substrates carrying a synthetic abasic site (a tetrahydrofuran modification). These substrates (AP substrates) were cleaved immediately 5' of the abasic site. Interestingly, in the presence of divalent cations (in particular Mg^{2+} and Mn^{2+}), NfoMpn and NfoMge demonstrated strong 3'>5' exonuclease activity on any double-stranded DNA substrate. When this activity was assayed using AP substrates, the cleavage of the AP sites was very rapid, and was followed by a slower, but progressive, digestion of the cleaved DNA.

Conclusion: The Nfo proteins from *M. pneumoniae* and *M. genitalium* have two distinct activities: (1) divalent cation-independent, specific cleavage of abasic sites in DNA, and (2) divalent cation-dependent 3'>5' exonuclease activity. These activities may play an essential role in the repair of damaged DNA in the bacterial life cycle.

P054

Rifampicin-induced transcriptome response in rifampicin-resistant *Mycobacterium tuberculosis*

G.J. de Knegt[1], O. Bruning[2], M.T. ten Kate[1], M. de Jong[2], A. van Belkum[3], H.P. Endtz[1], T.M. Breit[2], I.A.J.M. Bakker-Woudenberg[1], J.E.M. de Steenwinkel[1]

[1]Erasmus MC, MMIZ, Rotterdam, [2]University of Amsterdam, MAD-IBU, Amsterdam, [3]Biomérieux, R&D Microbiology, La Balme Les Grottes, France

Introduction: Tuberculosis (TB) is still a major life-threatening infectious disease, within which especially the rise of multidrug-resistant TB (MDR-TB) is currently worrying. This study focuses on mechanisms of development of rifampicin resistance, since this seems to play an important role in the development of MDR-TB.

Methods: To provide further insight in rifampicin resistance, we performed a genome-wide transcriptional profile analysis for *Mycobacterium tuberculosis* (*M. tuberculosis*) using micro-array technology and qRT-PCR analysis. We exposed a rifampicin-susceptible H37Rv wild type (H37Rv-WT) and a rifampicin-resistant progeny H37Rv strain with a H526Y mutation in the rpoB gene (H37Rv-H526Y) to several concentrations of rifampicin, to define the effect of rifampicin on the transcription profile.

Results: Our study revealed rifampicin resistance-dependant differences in response between both *M. tuberculosis* strains. Gene clusters associated with efflux, transport and virulence were altered in the rifampicin-resistant H37Rv-H526Y strain compared to the rifampicin-susceptible H37Rv-WT strain, after exposure to rifampicin.

Conclusion: We conclude that the small gene cluster Rv0559c-Rv0560c in the H37Rv-H526Y strain was remarkably up-regulated in the micro-array analysis and qRT-PCR results and appeared to be dependent on rifampicin concentration and time of drug exposure. Therefore this study suggests that Rv0559c and Rv0560c play a pivotal role in rifampicin resistance of *M. tuberculosis*. Further investigation of Rv0559c and Rv0560c is needed to reveal function and mechanism of both genes that were triggered upon rifampicin exposure.

P055

Bactericidal effect of bedaquiline (TMC207) and pyrazinoic acid on static mycobacterial cultures

P. Lu, H. Lill, A. Koul, D. Bald

VU Universtity, Dept. of Molecular Cell Biology, Amsterdam

Introduction: Discovery of new chemotherapy regimen is very import to combat the challenge of multi-drug resistant tuberculosis. Pyrazinoic acid (POA), the active moiety of the pro-drug pyrazinamide was previously reported that the bactericidal effect due to the depletion of cellular ATP reserves.[1]. The diarylquinoline bedaquiline (BDQ, TMC207), a new ATP synthase inhibitor[2,3], is the first drug approved by FDA in the last 40 years as a part of combination therapy to treat adults with multi-drug resistant tuberculosis.[4] Both drugs thus have effects on the mycobacterial energy metabolism, which has emerged as a new target-pathway for anti-tuberculosis drugs.[5] The combination of these two drugs displayed strong potency in mouse models.[6]

In order to gain insight in the mechanisms underlying this important synergy we investigate the combination treatment BDQ and POA for kill kinetics *in vitro*.

Methods: *In vitro* kill kinetic experiments were performed with static cultures of *M. bovis* BCG.

Results: In the employed static culture model system both BDQ and POA displayed bactericidal action. However, the bactericidal effect was significantly enhanced if the combination of the two drugs was applied. Under these conditions, bacterial counts reached the limit of detection within 3-4 weeks. This synergy observed in the kill kinetics correlated well with accelerated depletion of cellular ATP pools by this drug combination. Bacterial killing by BDQ was found enhanced in combination with the efflux pump inhibitor verapamil. We hypothesize that this synergism arises from depletion of cellular energy pools by BDQ or POA, indirectly inhibiting efflux pumps.

Conclusion: BDQ and POA synergize *in vitro*, most likely due to prevention of drug efflux caused by gradual depletion of cellular energy pools. The synergy previously observed in mouse models thus is not solely due to factors associated with the mammalian host. Since the combination of BDQ and POA was highly effective for killing persisting *M. tuberculosis*, these two drugs would seem to be an important component of new chemotherapy regimen aimed at reduction of the treatment period.

References

1) Lu P, et al. Antimicrob Agents Chemother. 2011;55:5354-7.
2) Andries, et al. Science. 2005;307:223-227.
3) Koul A, et al. Nat Chem Biol. 2007;3:323-4.
4) http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm333695.htm
5) Bald D, et al. FEMS Microbiol Lett. 2010;308:1-7.
6) Ibrahim, et al. Antimicrob. Agents Chemother. 2007;51:1011-1015.

P056

The Leiden CONCERT study – A randomized controlled trial with valganciclovir in infants with congenital CMV infection and sensorineural hearing loss

F.A.J. Schornagel, M.J. Korndewal, W. Soede, A.C.M. Kroes, J.J.C. de Vries, A.M. Oudesluys-Murphy, A.C.T.M. Vossen

Leiden University Medical Center, Dept. of Medical Microbiology, Leiden

Introduction: A previous randomized controlled trial with iv. ganciclovir has shown the efficacy of antiviral treatment in preventing deterioration of sensorineural hearing loss (SNHL) caused by congenital CMV. This study included newborns with established CNS disease. The objective of the CONCERT study (Congenital Cytomegalovirus: Efficacy of Antiviral Treatment in a Randomized

Controlled Trial) is to study the efficacy of valganciclovir in infants with solely SNHL.
Methods: All neonates routinely undergo neonatal hearing screening in the Netherlands during the first weeks after birth using Otoacoustic Emissions (OAE) technology. After the second refer an Automated Auditory Brainstem Response (AABR) screening is performed. The parents of all newborns (= 37 weeks gestational age) who fail this AABR (about 550 each year) will be asked to consent to CMV-testing on dried blood spots, obtained within 5 days after birth. Newborns diagnosed with congenital CMV and with confirmed SNHL (= 20 dB unilateral or bilateral) are eligible for inclusion. After informed consent infants will be randomized before the age of 13 weeks to a treatment group (6 weeks valganciclovir 32 mg/kg daily dose; oral solution) or a control group (no antiviral treatment). Infants will be monitored for leucopenia and liver- and kidney function. Inclusion will continue for at least 1.5 years, or until 25 infants have been randomized in each treatment arm.
Results: At 1 year follow-up hearing and general development will be assessed with brainstem evoked response audiometry. Hearing will be assessed at an audiological center, child development will be assessed with the Bayley Scales of Infant Development III during a home visit and parents will fill in the Dutch language Child Development Inventory (NCDI) which will give more detailed information on communicative development of their child. Viral loads in blood and urine will be monitored during antiviral treatment and twice in the control group.
Conclusion: This study will provide information on the percentage of infants who fail the neonatal hearing screening and who have a congenital CMV infection. The RCT will show whether early treatment of congenital CMV infected children with hearing impairment will prevent deterioration of hearing loss and to what extent. The outcome may lead to implementation of congenital CMV testing in the neonatal hearing screening program or possibly into the newborn blood screening.

P058

Protective human antibodies against multi-drug resistant *Staphylococcus aureus*

G. Buist[1], H.P.J. Bonarius[2], S. van den Berg[3], A. Dreisbach[1], M.M. van der Kooi-Pol[1], J.W. Back[4], K. van Kessel[5], J. van Strijp[5], H. Groen[2], I.A.J.M. Bakker-Woudenberg[3], J.M. van Dijl[1]
[1]*University Medical Center Groningen, Dept. of Medical Microbiology, Groningen,* [2]*IQ Therapeutics, Groningen,* [3]*Erasmus University Medical Center Rotterdam, Dept. of Medical Microbiology Infectious Diseseas, Rotterdam,* [4]*Pepscan Therapeutics BV, Lelystad,*[5]*University Medical Center Utrecht, Utrecht*

Introduction: *S. aureus* is an opportunistic community- and hospital-acquired pathogen that employs a great variety of cell wall-associated and secreted virulence factors to subvert its human host. With the ultimate aim to provide new strategies to combat life-threatening staphylococcal infections, it is the objective of the Top Institute Pharma project 'AntiStaph' to develop protective human antibodies for antimicrobial therapy against multi-drug resistant and highly virulent *S. aureus* strains, including MRSA.
Methods: A combination of proteomics, genomics, bioinformatics and immunological approaches was applied to identify conserved immunogenic determinants of relevant *S. aureus* isolates. Purified conserved proteins of *S. aureus* were used for the identification and isolation of antibody-producing B cells from peripheral blood of individuals, who are persistent *S. aureus* carriers. Monoclonal human antibodies were cloned and tested for specificity.
Results: Surface-exposed protein domains (the 'surfacome') of clinically relevant *S. aureus* strains have been identified by gel-free proteomics. Using peptide arrays, a detailed overview has been obtained of potentially relevant immunogenic epitopes in *S. aureus* surface proteins that are recognized by human antibodies. A first human monoclonal antibody, with high affinity for one of the conserved *S. aureus* surface proteins, has been identified and produced. This monoclonal antibody binds strongly to whole cells of different *S. aureus* serotypes and targets a factor important for growth. An immune complex of the monoclonal antibody with its target protein activates both mice and human neutrophils. Testing of the human monoclonal antibodies in a mouse model showed that mice were protected against lethal *S. aureus* infections at low dosages. Additional human monoclonal antibodies that bind staphylococcal antigens have been generated.
Conclusion: The isolated human monoclonal antibody binds all *S. aureus* serotypes investigated, targets factor essential for growth with high affinity, activates immune cells (neutrophils), protects mice against death due to severe *S. aureus* bacteraemia, and thus is a promising candidate for anti-staphylococcal therapy.

P059

Antimicrobial drug target in the emerging zoonotic pathogen *Streptococcus suis*

A.E.B. Bem, P. van Baarlen, J.M. Wells
Wageningen University, Animal Science, Wageningen

Two-component systems (TCSs) are used by bacteria to efficiently adapt to environmental changes such as temperature, osmolarity, chemoattractants and pH. They are the main system for signal transduction in bacteria. TCSs consist of a histidine kinase (HPK), which functions as a sensor, and a response regulator (RR), which regulates genes expression. The TCS YycF/G, conserved in a group of gram-positive bacteria which includes a number of important human pathogens, has been shown to be essential for these bacteria. YycF/G appears to regulate different sets of genes in each species although genes involved in cell wall metabolism are commonly part of the regulon. Moreover, YycF/G is often associated with the regulation of virulence. Therefore, it appears to be a main drug target for bacteria that are now only being controlled using antibiotics, such as *Streptococcus suis*. *S. suis* is a major bacterial pathogen of swine that can cause animal death which leads to high economical losses. *S. suis* is considered worldwide to be a problem for pig industry but it is also emerging as an important cause of meningitis in humans in South-East and East Asia.
The aim of this study is to reveal the structure and function of this putative essential TCS in *Streptococcus suis*. YycF, YycG and YycJ proteins and some of their protein domains were expressed using pTrcHis2 TOPO expression system. Proteins contained HisTag were purified by affinity chromatography. We are setting up bioassays to test various HPK inhibitors, to demonstrate phosphotransfer between HPK and RR and to find DNA binding motifs for RR. Upon obtaining the structure of YycF, YycG and YycJ proteins drug inhibition studies, docking studies and *in silico* drug design will be performed.

P060

A new real time PCR for rapid detection of VIM, OXA-48, NDM & KPC carbapenemases in *Enterobacteriaceae* directly from a rectal swab

R.H.T. Nijhuis[1], P.H.M. Savelkoul[2], A.A. van Zwet[1]
[1]*Rijnstate, Dept. of Medical Microbiology and Immunology, Velp,* [2]*MUMC+, Dept. of Medical Microbiology, Maastricht*

Objectives: The emergence and spread of drug resistance by bèta-lactamases amongst *Enterobacteriaceae* is a serious problem, in the hospital environment as well as in the community. Carbapenems have long been considered a solid last option for treating infections, caused by bèta-lactam resistant *Enterobacteriaceae*. However, since carbapenem resistance is emerging worldwide, due to the presence of various carbapenemase genes, treating infections with these multi-resistant strains has almost become impossible. In order to prevent the spread of these carbapenemases a fast and accurate carbapenemase detection in patient samples is extremely important. Moreover, since higher mortality rate is found in patients infected by carbapenemase-producing *Enterobacteriaceae*, rapid detection is even more essential. We describe the prototype of a novel multiplex real time PCR for rapid detection of the most common carbapenemases in *Enterobacteriaceae* (VIM, OXA-48, NDM and KPC) directly from rectal swabs, as a rapid screening approach.
Methods: A total of 53 gram-negative bacterial isolates with proven carbapenemase activity, along with 21 non-carbapenemase producers were used to evaluate this novel carbapenemase real time PCR. Furthermore, one carbapenemase producing *Escherichia coli* and three different carbapenemase producing *Klebsiella pneumoniae* isolates were used for spiking experiments in order to detect the analytical sensitivity of the assay, in comparison with culture using the ChromID CARBA agar.
Results: The carbapenemase real time PCR demonstrated an excellent performance of 100% (53/53) of the tested isolates with proven carbapenemase activity. All 21 non-carbapenemase producers were correctly identified as carbapenemase negative.
The limit of detection in rectal swabs was 0-0.5 CFU/PCR for VIM and OXA-48, 0.5 - 5 CFU/PCR for NDM and 5-50 CFU/PCR for KPC. All results were available within 3 hours (1 hour DNA extraction, 2 hours real time PCR).
Conclusion: This novel multiplex real time PCR is the first assay able to detect both VIM, OXA-48, NDM and KPC directly from rectal swabs. With excellent performance, this assay appeared to be an extremely accurate and sensitive method to detect carbapenemase genes VIM, OXA-48, NDM and KPC directly from a rectal screening swab within 3 hours.

P061

One disease, several patients and many *Staphylococcus aureus* types: high genetic diversity of nasal isolates from granulomatosis with polyangiitis patients

C.G. Glasner[1], M.M. van Timmeren[2], T. Stobernack[2], T.F. Omansen[1], E.C. Raangs[2], J.W. Rossen[2], J.P. Arends[2], G.A. Kampinga[2], W. van Wamel[3], A. Rutgers[2], C.A. Stegeman[2], C.G. Kallenberg[2], P. Heeringa[2], J.M. van Dijl[2]
[1]*University Medical Centre Groningen & University of Groningen, Dept. of Medical Microbiology, Groningen,* [2]*University of Groningen & University Medical Center Groningen, Pathology and Medical Biology, Groningen,* [3]*Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam*

Introduction: Patients suffering from granulomatosis with polyangiitis (GPA) are in 60-70% of cases chronic carriers of the opportunistic pathogen *Staphylococcus aureus*. In contrast, the *S. aureus* carriage rate amongst the healthy population is 20-30%. Nasal carriage of *S. aureus* is considered a risk factor for disease exacerbation in GPA patients, in particular if the carried *S. aureus* tests positive for superantigens. GPA, a severe autoimmune disorder, is an anti-neutrophil cytoplasmic antibodies (ANCAs) associated vasculitides (AAVs). The objective of this study was to assess the genetic diversity of *S. aureus* isolates from GPA patients to better understand the involvement of *S. aureus* in the onset and progression of GPA.

Materials and Methods: A total of 138 *S. aureus* isolates from 71 patients (41 males, age 56.315.3 y) isolated between 1990-1996 and 2006-2012 were subject to a combination of two typing methods, previously demonstrated to be a powerful approach for the typing of local and large numbers of clinical *S. aureus* isolates, namely multiple-locus variable number tandem repeat fingerprinting (MLVF) and spa-typing.
Results: Both methods clearly uncovered a high diversity of *S. aureus* isolates throughout the collection. The collection comprised a total of 71 distinct MLVF patterns and 45 different spa-types (including 5 unknown spa-types). With the application of a 70% cut-off value, the MLVF patterns showed the highest concordance (adjusted Rand's coefficient = 0.496) with the respective spa-types leading to 39 distinct MLVF clusters. Notwithstanding the high heterogeneity indicated by the determined spa-types and MLVF patterns, certain interesting trends were observed. Firstly, most of the 34 MLVF clusters that only comprised 1, 2, 3 or 4 isolates originated from one patient (i.e. one cluster per patient). Furthermore, the most frequent spa-types were t084 (n = 23, 13 patients), t064 (n = 22, 15 patients), t091 (n = 10, 6 patients), t012 (n = 9, 7 patients) and t021 (n = 8, 6 patients). Crucially, the isolates with the predominant spa-types also grouped together within the MLVF dendrogram. Intriguingly, the two predominant spa-types t064 and t091 were primarily identified amongst isolates from the period between 2006 and 2012, while the predominant spa-types t084 and t012 were solely identified in isolates from the period between 1990 and 1996. Out of 46 patients that provided > 1 isolate, 23 showed a shift in spa-types over time, whereas the remaining 23 carried the same spa-type over time. Intriguingly 5 of these patients presenting isolates with a shift in spa-types came from the two different time periods.
Discussion and conclusion: Altogether, the present data reveal for the first time that particular types of *S. aureus* may preferentially colonize GPA patients at a particular period in time. Overall, the diversity of *S. aureus* types colonizing patients with GPA is high, but the fact that certain predominant *S. aureus* types can be identified suggests that it may be possible to pinpoint particular staphylococcal traits that can be implicated in the onset and progression of GPA. Combined with the analysis of specific anti-staphylococcal immune responses of GPA patients, this might shed light on the impact of *S. aureus* on GPA.

P062
Identification and characterization of the replication machinery of *Clostridium difficile*

H.W. van Eijk
Leiden University Medical Center, Dept. of Medical Microbiology, Leiden

DNA replication is an essential and conserved process, and may therefore serve as a target for the development of novel antimicrobials. However, knowledge of the molecular biology and genetics of *Clostridium difficile* replication is very limited, compared to its well characterized close relative *Bacillus subtilis*. The identification and subsequent characterization of proteins involved in the initiation of replication can contribute to fundamental understanding as well as future clinical applications.
In the initial phase of our project, we focus on the proteins required for helicase loading in *Clostridium difficile*. The genes coding for these initiation proteins were identified on the basis of homology to replication genes of *Bacillus subtilis*. The putative replication proteins are investigated through biochemical and molecular methods, including (but not limited to) assessment of their DNA-binding activity and their ability to load the replicative helicase *in vitro*. We have purified the putative initiation protein (CD0001), a putative primosomal protein (CD3653) and the putative helicase loader ATPase (CD3654), as well as the helicase (CD3657) itself. These purified proteins are used for reconstitution of helicase loading *in vitro*. At this symposium, we will present the results from our ongoing experiments.

P063
Innate immune response against the human symbiont *Akkermansia muciniphila*

N.A. Ottman[1], T. Pietilä[2], A. Palva[2], H. Smidt[1], W.M. de Vos[1], C. Belzer[1]
[1]*Wageningen University, Laboratory of Microbiology, Wageningen,* [2]*University of Helsinki, Dept. of Basic Veterinary Medicine, Division of Microbiology and Epidemiology, Helsinki, Finland*

The recently discovered *Akkermansia muciniphila* is a strictly anaerobic, human intestinal bacterium, which colonizes a considerable part of the human population. It is abundantly present along the gut and can use mucin as a sole carbon and nitrogen source. *A. muciniphila* has been associated with a healthy intestine and numbers of *Akkermansia* in the gut inversely correlated with inflammatory bowel diseases, appendicitis, obesity and diabetes. Due to its close proximity to the epithelial cells, our studies focus on *A. muciniphila* host interactions and signalling pathways.
The proteins positioned on the surface of *A. muciniphila* are the first connection site to the mucus layer and these together with other cell-envelope components are potentially responsible for a host immune response. Bacterial cell fractionation techniques were established to isolate inner and outer membranes, surface-exposed proteins and secreted proteins. Enrichment for specific proteins was observed by SDS-PAGE, and LC-MS/MS analysis revealed candidate proteins for host recognition and immune stimulation Testing the proteome fractions on reporter cell lines indicated that NOD2-dependent NF-κB activation is induced by *A. muciniphila* outer membrane stimulation. This indicates that the surface-exposed proteins directly affect the host response. Also Toll-like receptor (TLR) 4- and TLR 2-reporter cells were activated by live and heat-killed bacteria, leading to interleukin-8 release by TLR 2-cells.
In conclusion, *A. muciniphila* is able to stimulate TLR 2, TLR 4 and NOD2-receptors *in vitro*, suggesting it has immunomodulatory properties. Our current research focuses on identification of bacterial proteins that activate the immune system. Further metabolic, proteomic and immune assays will shed more light on the interactions between *A. muciniphila* and the host.

P064
Detection of *Streptococcus pneumoniae* in nasopharyngeal samples underestimates pneumococcal carriage in the elderly

C.L. Krone[1], J. van Beek[2], N. Rots[2], M. Londja Akenda[1], A. Wyllie[1], M.L. Chu[1], D. Bogaert[1], E.A.M. Sanders[1], K. Trzcinski[1]
[1]*University Medical Center Utrecht, Pediatric Immunology and Infectious Diseases, Utrecht,* [2]*RIVM, Centre for Immunology of Infectious Diseases and Vaccines, Bilthoven*

Introduction: *Streptococcus pneumoniae* is a frequent but transient commensal of the human upper respiratory tract (URT). Since colonization of the URT is prerequisite to pneumococcal disease, and both high density and long duration of pneumococcal carriage are associated with pneumonia, there is growing interest in studying colonization dynamics. Pneumococcal disease, particularly pneumonia, disproportionally affects the elderly, however, detection of colonization in the elderly is rare (< 5%). Currently the gold standard for carriage detection in children is isolation of live *S. pneumoniae* from conventional culture of trans-nasally collected deep nasopharyngeal (TN) swabs; however, this method seems suboptimal for adults and an additional simultaneous trans-oral (TO) nasopharyngeal swab is recommended in order to increase sensitivity of carriage detection. Furthermore, switching from a culture dependent to a culture independent method of confirming the presence of pneumococci significantly increases method sensitivity. Interestingly, in the pre-antibiotic era, oral fluids were the primary specimens tested to detect carriage using a sensitive mouse inoculation method; with 45-60% colonization among healthy adults (Heffron, 1975). For this reason, we compared results of molecular detection of *S. pneumoniae* in TN and TO nasopharyngeal swabs and from saliva samples collected simultaneously from elderly individuals.
Methods: TN swabs, TO swabs, and saliva samples from 141 healthy elderly aged 60 to 90 years were cultured on blood agar plates, supplemented with 5 mg/l of gentamicin, and incubated overnight at 35 °C and 5% CO_2. Next, all bacterial growth was harvested into 10% glycerol in BHI and stored frozen at -70 °C. DNA was extracted from thawed raw and culture-enriched samples, using the Agowa protocol. Purified DNA was tested by quantitative-PCR (qPCR), targeting the pneumococcal autolysin (LytA) and ABC transporter (PiaA) genes. Samples were considered positive when CT values for both genes were < 40 in culture-enriched samples and < 45 in raw saliva samples.
Results: Saliva sampling was better tolerated by the elderly compared to swabbing of the nasopharynx as five persons refused TN and two refused TO swabbing, whereas saliva sampling was refused by none. Two of 137 (2%) culture-enriched TN, 7 of 140 (5%) culture-enriched TO, and 28 of 141 (20%) culture-enriched saliva samples were positive for *S. pneumoniae* by qPCR. The number of individuals positive for pneumococcus was significantly greater in culture-enriched saliva compared to either TN and TO samples (Fisher's exact test, $p < 0.001$). Of raw saliva samples tested, 25 (18%) were positive by qPCR including 16 positive also in corresponding culture-enriched saliva sample. All individuals with either a TN or a TO sample classified as positive for *S. pneumoniae* also had positive saliva samples by qPCR. Overall 37 (26%) elderly were positive for *S. pneumoniae* by qPCR in any of the 4 samples tested.
Conclusion: This study strongly suggests that use of only nasopharyngeal swabs to detect *S. pneumoniae* presence may greatly underestimate the colonization rates in the elderly population. In order to increase sensitivity of *S. pneumoniae* detection saliva and molecular methods should be considered when sampling the elderly for pneumococcal colonization.

P065
Assessing the pneumococcal serotype without culture in blood from patients with community acquired pneumonia using Capsular Sequence Typing

K. Elberse[1], S. Meijvis[2], C. Blauwendraat[3], I. van de Pol[1], L. Schouls[1]
[1]*National Institute for Public Health and the Environment (RIVM), Laboratory for Infectious Diseases and Screening (LIS), Bilthoven,* [2]*St Antonius Hospital, Dept. of Internal Medicine, Nieuwegein,* [3]*VUmc, Amsterdam*

Introduction: A century after its first description, the Quellung reaction still is the gold standard for serotyping of *Streptococcus pneumoniae*. The cost for the large number of antisera used by the Quellung reaction is high. Furthermore, in many cases a culture

of pneumococci, required for serotyping, is not available. As a result, an increasing number of pneumococcal infections are identified using DNA-based diagnostic methods on clinical material from patients. A molecular approach to assess the serotypes is therefore needed, reducing costs and increasing throughput and eventually the number of pneumococci being serotyped. Here we present an approach to use capsular sequence typing (CST) directly on blood samples to identify the serotype of the pneumococcus.

Methods: In CST part of one of the capsular genes of the pneumococcus is amplified and subsequently sequenced. Based on this sequence the serotype is assigned.[1] In this study, the CST protocol was adapted for use directly on blood samples. For PCR an internal control was added to detect inhibitors of amplification. To increase sensitivity of the assay the capsular gene segment was amplified in 2 smaller parts. Blood samples used in this study were obtained from patients above 18 years of age hospitalized with community acquired pneumonia (CAP) during the period of November 2007 to September 2010. Only blood samples positive for pneumococcus infection in the detection qPCR were used for serotyping with the clinical CST.

Results: Of the 304 included patients, we received 212 blood samples suitable to be used in this study. In total, 64 patients were positive for pneumococci using conventional methods to identify the causative agent of CAP, which included sputum and blood culture and urinary antigen test (BinaxNow *S. pneumoniae)*. Around 20% (n = 13) of these samples were also positive using the detection qPCR and 3 samples were qPCR positive but negative using conventional methods. We were able to assign a serotype to 11 of the 16 qPCR positive samples. The serotypes assigned in this study were serotypes 1 (n = 4), 3 (n = 3), 4 (n = 1), 6B (n = 1), 8 (n = 1) and 9V (n = 1). In the pneumococcal qPCR positive samples that were negative in the clinical CST, the bacterial load was less than 100 DNA copies per milliliter blood plasma.

Conclusion: Capsular Sequence Typing has proved to be a valuable method to assess the serotype of a cultured isolate. In this study, this assay was successfully adapted to be used directly on blood samples. In 70% of the samples positive for pneumococci with qPCR, a serotype could be assigned using the clinical CST.

References

1. Elberse, et al. PLoS One. 2011;6:e20390, www.MLVA.net.

P066

VIM-producing *Pseudomonas aeruginosa* in a long term care facility in the Netherlands: implications and practices

M. Scholing[1], P. Molenaar[2], E.B. Smit[3], R. Roosendaal[4], D. Notermans[5]

[1]GGD Laboratory for Public Health, Amsterdam, [2]GGD, Dept. of Hygiene and Infection Control, Amsterdam, [3]Wittenberg, Amsta, Amsterdam, [4]VUmc, Dept. of Medical Microbiology and Infection Control, Amsterdam, [5]National Institute for Public Health and the Environment (RIVM), Bilthoven

Carbapenamase-producing *Pseudomonas aeruginosa* are not primarily a problem in nursing homes, but will pose a threat when introduced in a hospital or intensive care unit (ICU). This report describes two cases of VIM-producing *P. aeruginosa* in a long term care facility. Currently there is no national surveillance for carbapenemase-producing *P. aeruginosa* strains in the Netherlands.

The first isolate was recovered from a urine sample of a 72 year old male resident, which was routinely taken upon removal of his urinary catheter. In the previous months he had been admitted twice to the surgical ward (first episode also to the ICU) of a nearby hospital for the treatment of Fournier gangrene and osteomyelitis of the right foot. As the patient was already admitted to a rehabilitation ward of a long term care facility for more than two months in a single-nursing room with a self-contained toilet, isolation precautions and contact tracing in the ward (18 residents) was only started after confirmation of VIM-production by the RIVM. As a result, a 78 year old male resident was found positive in a rectal swab for a *P. aeruginosa* with an identical antibiogram. VIM-production was again confirmed by the RIVM and amplified fragment length polymorphism (AFLP) typing revealed strong evidence for clonal expansion (AFLP type AT 12222). Isolation measures were instituted immediately and a second screening of contacts (including the neighbouring ward) was executed. Six months prior to admittance to the rehabilitation ward this second resident had also been admitted to the ICU and internal medicine ward of this nearby hospital for pneumosepsis. Their admissions, however, have not crossed in time room or ward.

In absence of tailor-made guidelines, the *Werkgroep Infectiepreventie* (WIP) guidelines for contact isolation in hospitals were applied to the outbreak ward of the long term care facility. In addition, the shared bathing facility was disinfected daily and preventive measures have been tightened. The second and third screening revealed no new carbapenemase-producing *P. aeruginosa* strains. Both initially positive residents tested negative in two follow up tests and within 2 months all isolation measures could be dissolved.

In 2011 and 2012 five laboratories in Amsterdam have sent 18 VIM-positive *P. aeruginosa* isolates to the RIVM for carbapenemase detection, of which eight from the hospital where the patients were admitted. Preliminary analysis of clinical data and AFLP typing results (AT 12120 and AT 12119) have not yet revealed a relation of these strains to the outbreak strain in the long term care facility.

To our knowledge, we describe the first detection of multiple VIM-producing *P. aeruginosa* in a long term care facility in the Netherlands. No further spread occurred with the application of WIP guidelines and tightening of preventive measures. Discussion is needed how to manage outbreaks of carbapenemase-producing bacteria in long term care facilities to prevent introduction in hospitals and ICU.

P067

TRAITOR study: 'Tuberculosis research on adjuvant immunotherapy through use of retinoic acid'

B.C. Mourik[1], G.J. de Knegt[1], M.T. ten Kate[1], S. Wang[2], P.J.M. Leenen[1], J.E.M. de Steenwinkel[1], I.A.J.M. Bakker-Woudenberg[1]

[1]Erasmus MC, Dept. of Medical Microbiology & Infectious Diseases, Rotterdam, [2]UC Berkeley, Nutritional sciences, Berkely, USA

Introduction: Tuberculosis (TB) kills 4700 people per day. The therapeutic efficacy of anti-TB drugs is complicated by the long treatment duration (> 6 months) and emergence of drug resistance. Our aim is to achieve shorter treatment duration with the current anti-TB drug regime by adding immunomodulating drugs that improve myeloid cell and NK-cell function.

Materials and Methods: Female adult BALB/c mice are intratracheally infected with 10^5 *Mycobacterium tuberculosis*.

Study 1. Four weeks after infection a 5 weeks treatment period is started with the standard anti-TB drug regime, consisting of isoniazid, rifampicin and pyrazinamide in human pharmacokinetic-equivalent doses, to which different combinations of immunomodulators are added. The immunomodulators used are all-trans retinoic acid (ATRA, myeloid cell modulator), calcitriol (myeloid cell modulator) and/or alpha-galactosylceramide (NK-cell activator).

At the end of treatment mice are sacrificed.

Parameters for effects of treatment:

- Tolerability of treatment.
- The immunomodulating effects of treatment on leukocytes in the broncho-alveolar lavage and bone marrow, assessed by flowcytometry analysis.
- ATRA serum concentrations.
- Decrease in *M. tuberculosis* load in infected organs (lung, spleen and liver).

Study 2. Four weeks after infection a 13 weeks treatment period is started with the standard anti-TB drug regime in combination with the optimal combination of immunomodulating drugs as found in Study 1.

At the end of treatment and 13 weeks post-treatment mice are sacrificed.

Ultimate outcome parameters for therapeutic efficacy:

- Elimination of *M. tuberculosis* in infected organs at the end of treatment.
- Prevention of relapse of infection at 13 weeks after termination of treatment.

Results: Our data on the optimization of the immunotherapy show that calcitriol and alpha-galactosylceramide are well tolerated in infected mice. However, TB infection causes a 2 to 3-fold increase in the serum ATRA concentration compared to non-infected mice and resulted in a decreased tolerability of ATRA in infected mice.

Discussion: The increase in serum concentration of ATRA is possibly caused by a TB-induced depression of the cytochrome p450 enzyme system, which is responsible for the metabolism of ATRA. Depression of this enzyme system has also been described for other infections. Therefore we will study the effect of TB infection on the cytochrome p450 enzyme activity in liver homogenate of infected mice. Next, once we have optimized the ATRA dosing, we will investigate the effects of adjuvant immunotherapy in TB treatment.

P068

Does the level of *Chlamydia* antibody titers (CAT) discriminate between fertile and infertile women?

J.C. van PeltKoops, K. Fleischer, F.F. Stelma

UMC St Radboud, MMB, Nijmegen

Background: The work-up of couples with fertility problems includes performing a *Chlamydia* antibody titer (CAT) of the female. Earlier CATs positivity has been shown to correlate with female infertility, however, it is unclear if high IgG levels increases the discriminatory value of the CAT-test. This retrospective study compares CAT titers from infertile women (tubapathology seen on hysterosalpingography (HSG) and laparoscopy) visiting a tertiary university hospital fertility clinic, to CAT titers of healthy fertile women. The objective of this study was to estimate a titer cut-off value for CAT-screening in the work up of infertile women.

Population and methods: Medical records of all women referred to the fertility clinic who underwent HSG and/or laparoscopy were included if CAT measurements were present (n = 409). Women were divided into tubal pathology negative or positive, according to HSG/laparoscopy findings. As healthy control group fertile pregnant women visiting a maternity out-patient clinic were included (n = 28) and CATs were determined retrospectively. In all patients CAT-index was determined by indirect semi-quantitative ELISA (Medac, Wedel, Germany). ANOVA and chi-square statistics were performed to compare geometric means (GM) of these groups to CAT-indices.

Results: Tubal pathology was found in 18% (61/333) of the infertile women who underwent HSG. In patients who underwent diagnostic laparoscopy, 35% (18/52) showed pathological findings (unilateral and bilateral).

No statistical differences were observed when comparing geometric mean CAT indices of patients undergoing HSG (GM index in HSG negative women 0,38; GM index in HSG positive 0,58, p > 0,05). There appeared to be a difference between fertile and infertile women as the GM index in fertile women was 0,12 (p = 0,026). Similarly, no significant differences were observed between the laparoscopy positive and negative groups, GM index respectively 1,32 and 0,58 (p > 0,05).
When considering the laparoscopic findings, and comparing CAT-indices between fertile and infertile women, the GM index in fertile women was 0,12 (p = 0,003).
When determining a CAT cut-off titer, an increased risk for tubal pathology was found above index = 1 considering both HSG (OR = 4.69 (1.3-17.2) and laparoscopic (OR = 8.33 (1.8 - 38,6). Higher cut-off values did not show statistical significant discriminatory properties.
Conclusion: The level of CAT index discriminates between fertile and infertile women, but does not discriminate between women with and without tubal pathology based on HSG and laparoscopy-findings. The cut-off value of 1 is in our laboratory the most appropriate cut-off value to predict tuba-pathology.

P069

Correlation between mucosal HPV 16/18 viral load and persistence in a cohort of young women in the Netherlands

A.J. King, E. van Logchem, J. Cremer, J. Sonsma, M. Mollers, M. Feltkamp, H.J. Boot
RIVM, CIb-Laboratory of Infectious diseases and screening, Bilthoven

Introduction: Infection with oncogenic human papillomavirus (HPV) is the main etiological factor in the development of cervical cancer. HPVs are nonenveloped small circularDNA viruses, of which 40 genotypes are known to infect the mucosal epithelium. HPVs are classified into two groups according to carcinogenic potential, high-risk (hrHPV) types (approximately 15 types including HPV16 and HPV18) being associated with lesions that can progress to cancer and low-risk types that cause lesions with little evidence for association cervical cancer. HPV appears to be the most common sexually transmitted infection and about 80% of all sexually active women will acquire an HPV infection during their lifetime. Fortunately, most HPV infections are transient and resolve within 2 years. Only persistent hrHPV infections, defined as two or more (type specific) HPVDNApositive tests with an interval of 6 to 12 months, are strongly associated with cancerous lesions. The HPV viral load reflects the productivity of viral replication and the number of infected cells, therefore its level may play a role in defining the course of HPV infections. The purpose of the study was to investigate if viral load of HPV16 and -18 predicted persistence of the HPV16/18 infection in young women.
Method: Vaginal self-swabs were collected in two rounds with an interval of one year in a prospective study conducted in the Netherlands among 3282 young women aged 16-29y. HPV16/18 viral load was assessed in the swabs by real time PCR targeting the L1 gene. To control for sampling heterogeneity, viral load measurements were normalized to human cells counts through the quantification of the human bèta-actin gene in the same sample. HPV DNA detection and genotyping was performed previously using the PCR-based reverse line blotting (SPF10-LiPA system version 1, DDL). Only women that were HPV16 and HPV18-positive at baseline and participated in two rounds were included in the analysis.
Results: Of the 233 HPV16- and 113 HPV18-positive women, 134 (57%) and 51 (45%) remained positive for the same HPV type during follow-up, respectively. Whereas 99 (43%) HPV16- and 62 (55%) HPV18- positive women had cleared the infection with in one year. Viral loads for HPV16 and HPV18 were determined at the first visit. The median viral load at first visit for cleared infections was 1.2 * 10^3 copies per million cells for HPV16, and 0.3 * 10^3 for HPV18. The median viral load at the first visit for persistent infections was 3.0 * 10^3 copies per million cells for HPV16 and 0.7 * 10^3 copies per million cells for HPV18.
Implications and impact: For both HPV16 and HPV18 the measured initial load was higher in women with persistent opposed to cleared infections. For HPV16 statistical significance was reached (p = 0.0057, Mann-Witney test). Our findings suggest a correlation between initial HPV16 and HPV18 load and persistence of infection.

P070

No additional value of throat swabs in the diagnosis of enterovirus infections

M.M. Jager, S.J. Vainio, C.W. Ang
VU University Medical Center, Dept. of Medical Microbiology and Infection Control, Amsterdam

Introduction: Demonstration of enterovirus (EV) RNA in cerebrospinal fluid is definite proof of EV meningitis. The presence of EV RNA in other locations such as throat or feces, in a patient with sepsis or meningitis is also considered to be a strong indication of EV as the cause of the disease episode. It is currently unknown what the additional value is of sampling from other sites such as throat or feces. However, the use of PCR in the diagnostics is relatively expensive and unnecessary testing should therefore be avoided. This study aims at defining the additive value of testing throat and/or fecal samples for diagnosis of enterovirus infections.
Methods: We analyzed samples that were tested with an in-house real-time PCR for EV between the 1st of January 2007 and the 1st of October 2012. In total, 566 patients had one or more samples tested for EV, 322 of them were below the age of one year. We used a composite reference standard with the following definition: patients that were positive in any of the PCR's for CSF, throat or feces were regarded as 'EV-positive'. Samples that were inhibited in the PCR were regarded as negative results.
Results: The frequencies of a positive PCR in CSF, throat and feces were 16%, 25% and 20% respectively. De sensitivity of testing 'CSF only' was 86%, for 'throat only' 75% and for 'feces only' this was 96%. We also investigated whether combinations of PCR's from multiple sites were more informative. In the group of patients with both CSF and throat swab, a small group had a negative CSF PCR but a positive throat PCR. However, in all patients that had also their feces tested, the feces PCR was positive, indicating that a throat PCR does not provide extra information when a feces PCR is performed. In the group of patients with both CSF and feces PCR, we observed that a substantial number of patients had a positive feces PCR and negative CSF PCR.
In children below the age of 1, frequencies of positive PCR's were significantly higher than for older patients. In this subgroup, the results from combinatorial testing were similar as for the whole group.
Conclusion: Our data show that including a throat swab in the diagnosis of EV infection has only limited additional value and can be omitted. This will lead to a reduction in costs, which is very important in the current economic situation, without compromising sensitivity.

P071

Comparison of three *Plasmodium falciparum* isolates in a controlled human malaria infection

M.B.B. McCall[1], L.J. Wammes[1], C.C. Hermsen[2], R. Koelewijn[3], S. Chishimba[1], S. Goeijenbier[1], N. Molhoek[3], M. IJsselstijn[3], G.J. van Gemert[2], W. Graumans[2], M. van de Vegte[2], H.A. Verbrugh[1], J.J. van Hellemond[1], P.J.J. van Genderen[3], R.W. Sauerwein[2]
[1]Erasmus MC, Dept. of Medical Microbiology & Infectious Diseases, Rotterdam, [2]UMC St. Radboud, Dept. of Medical Microbiology, Nijmegen, [3]Havenziekenhuis, Dept. of Parasitology, Rotterdam

Background: Recent years have witnessed major breakthroughs in the development of a protective (attenuated whole-parasite-based) malaria vaccine. As a next step, such vaccines' cross-protective efficacy against diverse global parasite strains must be determined. Here we have assessed two native *P. falciparum* isolates alongside a well-characterised laboratory strain in a controlled human malaria infection, in order to determine their suitability for use in upcoming heterologous challenges studies of malaria vaccines.
Methods: Three groups of five healthy malaria-nave adult volunteers were each infected with one of three *P. falciparum* strains by mosquito bite. As soon as thick smear positive, subjects were treated with a curative regimen of Malarone. Clinical and parasitological course of infection were assessed, the latter retrospectively by QRT-PCR.
Results: All fifteen volunteers became thick-smear positive between day 6.5 and 10.5 post-infection and were curatively treated. The time to thick smear positivity for the two native isolates, both 7.0 (6.5-8.5) days (median (range)), was shorter than for the laboratory strain, 10.5 (9.0-10.5) days, p < 0.05. The clinical course was generally mild-moderate and did not differ significantly between strains, with severe adverse events occurring in only 4/15 subjects (two with temperature > 39.0 °C and two with severe malaise); no serious adverse events occurred. Sub-microscopic parasitaemia, as measured by QRT-PCR, displayed a characteristic 48-hour cyclical multiplication pattern following release from the liver, with the initial wave peaking at day 7.0 post-infection in all strains.
Conclusion: The two native *P. falciparum* strains generate robust and safe infections in human volunteers, allowing their impending use in crucial heterologous challenge studies of candidate malaria vaccines.

P072

Helicase loading in *Clostridium difficile*: investigating interactions between replication initiation proteins

A.H. Friggen, H.W. Eijk, W.K. Smits
LUMC, Dept. of Medical Microbiology, Leiden

DNA replication is an essential and conserved process, and may therefore serve as a target for the development of novel antimicrobials. However, knowledge of the molecular biology and genetics of *Clostridium difficile* replication is very limited, compared to its well characterized close relative *Bacillus subtilis*.
In the initial phase of our project, we focus on the proteins required for helicase loading in *Clostridium difficile*. The genes encoding these initiation proteins were identified on the basis of homology to replication genes of *Bacillus subtilis*. Although different organisms use different mechanisms for helicase loading, it is clear that it is a hierarchical process involving numerous initiation proteins. The putative initiation protein (CD0001), a putative primosomal protein (CD3653) and the putative helicase loader ATPase (CD3654), as well as the helicase (CD3657) itself have been purified. Furthermore, antibodies have been raised against these purified proteins. Protein-protein interactions between initiation proteins are investigated through a multifaceted approach involving

cross-link-, two-hybrid and pull-down experiments. At this symposium, we will present the results from our ongoing experiments.

P073

Group A *Streptococcal* outbreak among patients receiving out of hospital wound care

V. Hira[1], K. Heijman[2], A.T.P. Brink[1], M. Theunissen[1], E. Smeets[1], S. Herngreen[1], K. van der Zwaluw[3], D.W. Notermans[3], M. Heck[3], M. Dreessen[4], E. de Brauwer[4], C. Hoebe[2], P. Wolffs[1], C. Linssen[4], E. Stobberingh[1]
[1]MUMC, Dept. of Medical Microbiology, Maastricht, [2]Public Health Service South Limburg, Dept. of Sexual Health, Infectious, Geleen, [3]National Institute for Public Health, LIS/CIb, Bilthoven, [4]Atrium Medical Center Parkstad, Dept. of Medical Microbiology, Heerlen

Introduction: Group A Streptococci are a major cause of outbreaks. From January to May 2012, we observed an increase in cultures of Group A Streptococci (GAS) at the Maastricht University Medical Centre. Analysis showed that this was attributable to an increase in GAS from infected wounds. We aimed to identify possible causal factors.
Methods: Patient information was obtained from hospital medical charts. All GAS isolates were subjected to emm-typing, and pulsed field gel electrophoresis (PFGE).
Results: From January to May 2012, GAS was cultured from 33 patients. In 22 of these, the GAS was cultured from an infected wound (outbreak patients). These 22 patients had no common factors besides receiving home wound care home from company X prior to wound infection. Eighteen (82%) had received negative pressure therapy (NPT). Work charts of the wound care nurses showed no relation in time with the outbreak patients. Two of the nurses and one NPT pump were positive for GAS.
Seventeen outbreak isolates were available for molecular typing, eleven were emm type 89.0, six were emm-type 75.0. PFGE showed that all emm-type 89.0 isolates belonged to a PFGE cluster (A) and all emm-type 75.0 isolates belonged to PFGE cluster B. One GAS isolated from the wound care nurses was available for analysis and was emm-type 75.0, PFGE B. The GAS isolated from the NPT pump was emm-type 89.0, PFGE A.
Conclusion: The increase in GAS was attributable to a clonal outbreak among wound infection patients who had received out of hospital wound care by company X. No causal relationship and no risk factors could be identified. As there was no registration of the NPT pumps, they could not be related to patients. Due to our findings the company set up a registration system for all NPT pumps and send the pumps to the manufacturer for thorough cleaning after each patient treatment.

P074

Staphylococcus epidermidis and Staphylococcus aureus, the major causing agents of biomaterial-associated infection, induce dendritic cell maturation

P. Balraadjsing, E.C. de Jong, S.A.J. Zaat
Academic Medical Center, Dept. of Medical Microbiology, Amsterdam

Introduction: *Staphylococcus epidermidis* and *Staphylococcus aureus* are the major pathogens in medical device (biomaterial)-associated infection. These bacteria can survive in biofilms on the biomaterial and are not efficiently cleared form the tissue surrounding the biomaterial. In these biomaterial-associated infections the local immune response to the bacteria is impaired. The combination of a biomaterial and bacteria provokes unexpected inflammatory reactions. The nature of these reactions is dependent on the physicochemical characteristics of the biomaterials and on the immune activating potential of the bacteria. A major cell type orchestrating these immune responses are dendritic cells (DCs). To fully understand these responses, insight needs to be obtained into maturation of DCs induced by staphylococci, by biomaterials and by the combination.
Aim: The aim of this study is to asses how *S. epidermidis* and *S. aureus* induce DC maturation, as a first step to understand the immune regulation involved in biomaterial-associated infection.
Methods: Monocyte-derived DCs were cultured with *S. epidermidis* or *S. aureus*, or with LPS as a positive control. Cell surface maturation markers HLA-DR, CD86 and CD83 were measured after 48 h of incubation, by FACS analysis. In separate experiments, TNF-α, IL-10, IL-6, IL-23, IL-12p70 and IL-1 cytokine levels were measured in supernatants collected after 24 h of incubation. The experiments were performed 4 times, with DCs of different donors.
Results: The expression of HLA-DR, CD86 and CD83 was increased upon co-culture of DC with *S. epidermidis* as well as with *S. aureus* in a bacterial concentration-dependent fashion. DCs matured with *S. epidermidis* or *S. aureus* did not secrete detectable levels of IL-12p70 or IL-1. *S. aureus* induced the production of higher levels of TNF-α and IL-10 than did *S. epidermidis* for most of the donors. The production of IL-6, IL-23, IL-12p70 and IL-1 was similar after stimulation with both bacteria, but showed relatively large interdonor variation.
Conclusion: DCs are activated to a similar extent by *S. epidermidis* and *S. aureus* as judged from the expression of maturation markers and secreted cytokines. In future research we will investigate the effect of the combined presence of staphylococci and biomaterials on DC maturation.

P075

Population structure of *Staphylococcus aureus* isolates in Europe and China by multiple locus variable number tandem repeat analysis

X. Yan[1], L.M. Schouls[2], G.N. Pluister[2], X. Tao[3], X. Yu[4], Y. Song[5], F. Luo[5], J.M. van Dijl[1], J. Zhang[3], H. Grundmann[1]
[1]University Medical Centre Groningen, University of Groningen, Dept. of Medical Microbiology, Groningen, [2]National Institute for Public Health and the Environment (RIVM), Bilthoven, [3]National Institute for Communicable Disease Control and Prevention, Beijing, China, [4]Heilingjiang province Center for Disease Control, Harbin, China, [5]Chaoyang Center for Disease Control and Prevention, Beijing, China

Introduction: *Staphylococcus aureus* is one of the most important human pathogens due to its virulence potential and ubiquitous occurrence as a colonizer in humans, domestic animals, and livestock. The aim of this research was to compare the population structures of *S. aureus* circulating in Europe and China in order to better understand evolution and global spread of this pathogen.
Methods: A collection of 791 *S. aureus* isolates (255 clinical, 394 healthy carriage, 138 pig and 4 food isolates, 85% MSSA, 15% MRSA) representing the extant and natural population of *S. aureus* in various locations in China was characterized. For comparison, the same number of *S. aureus* isolates (791 clinical, 63.2% MSSA, 36.8% MRSA) was randomly selected from a European Staphylococcal Reference Laboratory (SRL) survey performed in 2006-2007. All isolates were characterized by multiple locus variable number tandem repeat analysis (MLVA).
Results: Among the 791 Chinese isolates 272 MLVA types were identified clustering into 16 distinct MLVA complexes (MCs), including 114 MLVA types (16 MLVA complexes) from clinical samples, 173 MLVA types (16 MLVA complexes) from healthy carriage, 15 MLVA types (3 MLVA complexes) from pigs and 2 MLVA types (1 MLVA complex) from food. In the European collection, 422 MLVA types were clustered into 23 MLVA clonal complexes. MC8 and MC5 were the predominant hospital-associated (HA) MRSA clades in China representing 59.6% and 28.1% of all MRSA isolates; while MC5, MC8, MC22 and MC2 were the main HA-MRSA clades in Europe, altogether accounting for 80% of all MRSA isolates. MSSA populations were more heterogeneous. MC5, MC7, MC437, MC8, MC1 and MC398 accounted for around 50% of all MSSA isolates among clinical samples in China, while MC30 and MC45 were the predominant clades in the European collection accounting for 14.2% and 13.8% of all MSSA isolates respectively. MC398 was the predominant MSSA MLVA complex among both healthy carriers and pigs in China. Two MLVA complexes (MC398 and MC7) were represented by isolates from patients, healthy carriers and pigs, suggesting some degree of exchange of strains between human and animals in China. Based on phylogenetic inference (minimum spanning trees), the founders of MC398 and MC9 seem to have emerged in China whereas those for MC22, MC45 and MC30 more likely originate from Europe. Moreover, an ancestral relationship between MC45, MC398 and a newly emerging Chinese HA-MRSA MC2147 seems likely.
Conclusion: 1. There seems to be a systematic difference in the distribution of *S. aureus* clades between Europe and China.
2. Minimum spanning trees showed that some clades representing successful HA-MRSA in Europe hardly exist in China and *vice versa*.
3. These results will help to gain insights about the geographical and temporal emergence of clades with particular public health importance and to better understand the evolution of *S. aureus* as a whole.

P076

Lipopolysaccharide stimulation inhibits viral infection of human macrophages with two distinct mechanisms

M. Vissers[1], K. Short[2], P.W.M. Hermans[1], D. Diavatopoulos[1], G. Ferwerda[1]
[1]RUMC, Laboratory of Pediatric Infectious Diseases, Nijmegen, [2]The University of Melbourne, Dept. of Microbiology and Immunolog, Melbourne, Australia

It is well known that respiratory viral infections enable commensal bacteria in the nasopharynx to disseminate to other sites in the body and cause disease. However, this is not a uni-directional interaction and the presence of specific bacterial species in the nasopharynx may also affect viral pathogenesis. At present, it remains unclear if bacteria are able to exacerbate viral infections or if bacteria possibly serve to limit viral infections. We use influenza A virus (IAV) and respiratory syncytial virus (RSV) to examine how bacterial ligands in the nasopharynx can affect the pathogenesis of subsequent viral infections.
Human monocyte derived macrophages are cultured from buffy coats or blood from healthy volunteers. The macrophages are stimulated with bacterial ligands after which they are infected with GFP-IAV and GFP-RSV. Flow cytometry is used to determine percentage infection and phenotyping of the macrophages.
Human epithelial cells (A549) stimulated by a panel of different bacterial ligands did not display any significant alteration in susceptibility to RSV or IAV. However, pre-stimulation of human monocyte derived macrophages with lipopolysaccharide (LPS) reduced the infection rate of both RSV and IAV with approximately 80%. In contrast, bacterial ligands derived from gram-positive bacteria (lipoteichoic acid (LTA) and muramyl dipeptide (MDP))

did not inhibit viral infection. The protective effect was not due to pro-inflammatory cytokines or apoptosis of the macrophages. Our results show that LPS protects against viral infection in two distinct mechanisms. LPS inhibits the entry of RSV early in the infection, whereas, in contrast, it triggered a long-term, non-specific, type I interferon response to prevent IAV infection.
Taken together, these data demonstrate the bi-directional nature of viral-bacterial interactions, and that the composition of an individual's nasopharyngeal flora may help determine their susceptibility to viral infections.

P077

Quality control for routine viral molecular diagnostics using viral culture techniques

P.M. van Gijssel, H. Meijer, M.T. de Walle-Bolhuis, M. van Oosten, D.S. Luyt, C.A. Benne
Laboratorium voor Infectieziekten, Medische microbiologie, Groningen

Introduction: Since December 2011, virtually all routine viral detection in the Laboratory for Infectious diseases in Groningen was based on polymerase chain reaction (PCR). However, the PCR-based detection and identification of viruses assumes a known, relatively stable genome. Unfortunately, high mutation rates may lead to extensive changes in viral nucleic acid sequences which may hamper detection by dedicated PCR primers. To understand the impact of this potential problem for routine diagnoses, we introduced a quality control by testing a selection of PCR negative samples with viral culture.
Methods: Retrospectively, 110 frozen (-80 °C) respiratory samples were selected in the period of week 49 of 2011 to week 13 of 2012. The selection was based on a negative viral PCR and the presence of clinical symptoms. This selection had an even distribution of gender and age compared to all respiratory samples in the same period. The samples were inoculated on shell vials with Madin-Darby Canine Kidney Epithelial Cells (MDCK Line) for influenza viruses, human epidermoid carcinoma cells (HEP_2) for respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) and adenovirus (ADV), monkey (*Macaca mulatta)* kidney cells ($LLc.MK_2$) for parainfluenza viruses and human embryonic lung cells (HEL) for rhinovirus. The cultures were tested by immunofluorescence (IF) on day 1, 3 and 7 using commercial monoclonals directed to ADV, RSV, hMPV, influenza A, B, parainfluenza 1, 2, 3 and a pan-entero monoclonal (oxoid and millipore). 63 Samples were tested for Influenzavirus A, B and RSV and 47 samples for Influenzavirus A, B, RSV, parainfluenza virus 1, 2, 3, ADV, hMPV, rhinovirus.
Retrospectively, 41 frozen (-20 °C) stools were selected in the period of week 27 to week 39 of 2012. The selection was based on a negative PCR for ADV, enterovirus and parechovirus and the presence of clinical symptoms. This selection had an even distribution of gender and age compared to all stools in the same period. The samples were tested by viral culture using HEL and human colorectal adenocarcinoma cells (CaCo-2 and HT29). Viral cultures were examined microscopic every 3 days for a total of 14 days. Suspected cultures were tested by shell vial culture and IF using a pan-entero monoclonal and an ADV monoclonal.
Results: All respiratory and stool samples were negative by shell vial culture or by viral culture for the mentioned analyzed viruses.
Conclusion: In the tested periods, no new variant virus was found by viral culture techniques, that was missed by routine PCR. However, we only tested one viral season for respiratory viruses and enteric viruses. So we have too little data to make a definite conclusion. We will continue testing viral PCR negative samples as a quality control. We will inform you about our findings.

P078

Selective enrichment broth or regular ESwab for the detection of MRSA by PCR?

M.J.A. Knops, E.I.G.B. de Brauwer, A. Schopen-Waelen, F.S. Stals, C.F.M. Linssen
Atrium MC, Dept. of Medical Microbiology, Heerlen

Introduction: Rapid screening for the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) may decrease the time of strict isolation leading to reduced costs and increased patient welfare.
The aim of the present study was to compare two types of broths in combination with an ESwab for the molecular detection of MRSA in screenings samples.
Materials and method: Previously MRSA positive patients were screened using two sets of ESwabs (inserted in either liquid amies medium (E-LAM) or selective enrichment broth (TSB Salt Broth (E-TSB), Copan, Brescia, Italy). Samples were collected from nose, throat, perineum and wounds. For DNA isolation 200 l of the samples was lysed with achromopeptidase (Becton Dickinson BV, Breda, the Netherlands). PCR was performed using the BD GeneOhm MRSA ACP Assay (Becton Dickinson BV, Breda, theNetherlands). Additionally, culture using enrichment broth was performed for both E-LAM as E-TSB.
Results: Out of 107 samples (33 patients) included, 31 samples (14 patients) showed positive results (PCR and/or culture). Out of these 31 samples, 16 were positive in both the E-LAM and the E-TSB group, whilst 7 were negative in both groups. Furthermore, 8 samples were additionally positive in either the E-LAM (4 samples) or the E-TSB group (4 samples). A total of 7 samples were PCR negative whilst culture showed the presence of MRSA. In 2 samples, PCR was positive whilst culture remained negative, both samples belonged to the E-LAM group and showed high Ct-values (Ct 39.52 and 37.31). The overall agreement between PCR and culture was 92% for the E-LAM group versus 94% for the E-TSB group. The agreement between E-LAM en E-TSB was 93%.
Conclusion: Additional studies are necessary to evaluate the effect of increasing the input of isolated DNA on the PCR results.
The 2 PCR positive, culture negative results may be false positive, additional tests are necessary to confirm or disprove these results. Sampling error may be (one of the) causes for the discrepancies in PCR results between the E-LAM and the E-TSB group. Culture using enrichment broth is the most sensitive method for the detection of MRSA.
No significant difference was found in identifying the presence of MRSA by means of PCR using either ESwab inserted in Liquid Amies Medium or the ESwab inserted in TSB Salt Broth.

P079

Selective enrichment broth or regular ESwab for the detection of MRSA by culture?

M.J.A. Knops, C.F.M. Linssen, A. Schopen-Waelen, F.S. Stals, E.I.G.B. De Brauwer
Atrium MC, Dept. of Medical Microbiology, Heerlen

Introduction: Screening for the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) prevents the spread of this potential pathogenic micro-organism. State of art diagnostics in our laboratory include the use of ESwab, additional incubation in enrichment medium and finally the use of chromogenic agar plates. Currently the ESwab is inserted in enrichment medium whilst commercial ESwab with MRSA enrichment medium is offered as a package.
The aim of the present study was to compare the conventional ESwab with the ESwab with enrichment medium (TSB Salt broth) for the detection of MRSA in screenings samples.
Materials and method: Previously MRSA positive patients were screened using two sets of ESwabs (inserted in either liquid amies medium (E-LAM) or selective enrichment broth (TSB Salt Broth (E-TSB), Copan, Brescia, Italy). Samples were collected from nose, throat, perineum and wounds. E-LAM were inserted in aztreonam-tryptosefosfate broth (Tritium Microbiology, Eindhoven, the Netherlands) E-TSB were immediately incubated at 37 for 18-24 hours. After incubation 10l of the cultured broth was inoculated on MRSA-ID agar (Biomerieux, Marcy l'Etoile, France) by a walk away specimen processor (WASP, Copan, Brescia, Italy).
Results: Out of 107 samples (33 patients) included, 29 samples (14 patients) showed positive culture results. Out of these 29 samples, 21 were positive in both the E-LAM and the E-TSB group. Furthermore, 3 samples were additionally positive in the E-LAM and 4 additional samples showed the presence of MRSA in the E-TSB group. The agreement between E-LAM en E-TSB was 94%. Since culture results did not reach an agreement of 100%, patients may be misdiagnosed as MRSA negative. In the current study one patient was misdiagnosed as MRSA negative in the E-LAM group. Discrepancies may be the result of sampling error and depend on which ESwab was used for collection first.
Conclusion: Sampling error may be (one of the) causes for the discrepancies in culture results between the E-LAM and the E-TSB group.
No significant difference was found in identifying the presence of MRSA by means of culture using either ESwab inserted in liquid amies medium or the ESwab inserted in TSB salt broth.
The use of the ESwab in TSB salt broth fits within the Lean-management principle since no additional step (introducing the ESwab in the enrichment medium in the laboratory) is needed.

P080

Metagenome reconstruction of nasopharyngeal microbiota of young children based on 16S rDNA sequence data

W.T. Hendriksen, X. Wang, G. Biesbroek, K. Trzcinski, E.A. Sanders, D. Bogaert
UMC Utrecht-WKZ, Dept. of Pediatric Infectious Diseases and Immunology, Utrecht

Introduction: A balanced nasopharyngeal (NP) microbiota is likely to play a key role in human health, keeping pathogens out and opportunists at bay. However, in a disturbed NP microbiota (e.g., due to viral infection, immunization or antibiotic treatment), these pathogens or opportunists might have the chance to emerge and cause disease. Studying the underlying bacterial interactions will give us insight in which conditions are favorable for a balanced, and hence, healthy microbiota. Low density of microbial communities of the upper respiratory tract makes full genome metagenomic sequencing virtually impossible. Here, we aimed to develop a method to predict the microbiota gene content based on 16S rDNA data in order to identify differences in genes and gene pathways significantly correlating with metadata, i.e., age, viral infection, antibiotics usage, PCV7 vaccination (the pneumococcal vaccine) status, patterns of social and family contacts.
Method: We investigated the NP bacterial composition of 200 children, sampled at the age of 12 and 24 months, using 454-pyrosequencing of the 16s rDNA gene V5-V6 hyper variable region (Bogaert et al, 2011). The microbiota profiles consisted predominantly of three bacterial species; *Streptococcus pneumoniae, Moraxella catarrhalis,*

and *Haemophilus influenzae*. Using R software with the KEGGSOAP package (that connects with the online database KEGG (www.kegg.com)), we reconstructed gene pathways of the most dominant bacterial genera present in the nasopharyngeal microbiota. We calculated relative gene frequencies by multiplying the presence of the bacterial genera in a sample with the presence of a gene of that genus in the KEGG database. Only samples that had at least 95% of all the 16S rDNA reads assigned to a genus present in the KEGG database were used for this reconstruction. Subsequently, we applied Mann-Whitney U testing to find significant differences in relative gene frequencies between groups of samples, which were grouped based on metadata (age, antibiotics usage, vaccination status, daycare attendance, patterns of social and family contacts). In order to confirm that particular pathways are unique for a given metadata profile, genes that differed significantly (p-value < 0.005) were further analyzed by MinPath (Ye and Doak, 2009). It allowed us to retrieve the minimal amount of pathways sufficient to describe the genes that were analyzed.
Results and conclusion: Using our method, we were able to observe genes in several gene pathways enriched (or reduced) in 12 versus 24-month old children (age-dependent differences), in children treated with antibiotics or infected with a virus. Vaccination with PCV7, or known social and family contacts did not show a correlation with gene frequencies.
Our method provides a relatively quick way of analyzing the putative gene content of low density microbiota, providing a fast screen to identify potentially differently present gene pathways in microbiota in relation to metadata profiles.

P081

Long-term trends in incidence and resistance of fecal bacterial pathogens in the Netherlands

M.P.D. Deege[1], M.J.M. Bonten[2], E. Talboom-Kamp[3], J.G. Kusters[2]
[1]*Saltro/UMCU, Medische microbiologie, Utrecht,* [2]*UMCU, Medische microbiologie, Utrecht,* [3]*Saltro Diagnostisch Centrum, Utrecht*

Introduction: *Salmonella enterica*, *Shigella*, *Yersinia* and *Campylobacter* spp. (SSYC) are important causes of bacterial diarrhea. Antibiotic treatment is not recommended for these infections, unless patients are very ill or immunocompromised. We determined longitudinal prevalence of SSYC and antibiotic susceptibilities for commonly used antibiotics in stool samples submitted by general practitioners in the Netherlands over the last ten years.
Methods: Our lab services 677 GPs (7.6% of all GPs in the country) and is located in the middle of the Netherlands. All fecal samples (n = 50,234) submitted for SSYC culture from 1-1-2002 to 31-12-2011 were included. Conventional culture techniques were used, and susceptibilities were determined by agar diffusion for *Campylobacters* and by an automated system (VITEK2) for the other bacteria. Breakpoints were based on CLSI criteria.
Results: Numbers of fecal samples per year ranged from 4671 (2007) to 5586 (2004), and fractions of samples with growth of SSYC ranged from 6.6% (2008) to 9.0% (2005), with the following prevalences per species: *Campylobacter* spp. 4.5-6.9%, *Salmonella enterica* ssp. 1.1-2.0%, *Shigella* spp. 0.1-0.3% and *Yersinia* spp. 0.04-0.17%. Co-trimoxazole resistance was below 15% for *Salmonella* and *Yersinia* and ranged from 70-100% until 2008 for *Shigella* and declined to 63% in 2009, 69% in 2010 and 33% in 2011. Ciprofloxacin resistance was over 55% for *Campylobacter* and increased in *Salmonella* and *Shigella* from 0% until 2005, to over 14% in 2011. Macrolide resistance in *Campylobacter* was stable from 2002-2007 (0.6%-1.4%) but increased to over 2% in 2009-2011.
Conclusions: Bacterial pathogens were cultured in 7-9% of stool samples submitted by GPs. Resistance to ciprofloxacin increased for *Salmonella* and *Shigella* and to macrolides for *Campylobacter*. Resistance to co-trimoxazole decreased in *Shigella*.

P082

Neutrophil-lymphocyte count ratio: a promising selection marker for molecular diagnostics of patients suspected of having bacteremia

A.J.M. Loonen, J. Tosserams, P. de Jager, P. Wever, A.J.C. van den Brule
Jeroen Bosch Ziekenhuis, Molecular Diagnostics, 's-Hertogenbosch

Introduction: Bacteremia is a serious medical condition. Fast and accurate identification of the etiologic pathogen is therefore of clinical importance. Sensitive molecular analysis of bacteremia using large blood volumes is currently available. However, the high costs of molecular tests to date require a selection marker, which can preselect patients for immediate molecular testing besides blood culture.
Methods: One ml residual EDTA blood was obtained from 140 patients presenting at the Emergency Department with = 2 SIRS criteria. Selective pathogen DNA isolation was performed with Polaris (Biocartis) and MolYsis (Molzym), and samples were analyzed for the presence of pathogens using both the commercially available Magicplex Sepsis test (Seegene) and SepsiTest (Molzym). Additionally, C-reactive protein (CRP), neutrophil-lymphocyte count ratio (NLCR), procalcitonine and soluble urokinase plasminogen activator receptor (suPAR) levels were obtained.
Results and Discussion: Eleven patients were excluded from the study for several reasons (129 patient samples remaining). The evaluation of patient samples resulted in 29/129 positive blood cultures (22.5%), with *E.coli* detected most frequently (n = 10). Molecular diagnostics of pathogens is presently ongoing, as well as the analysis of the markers for molecular diagnostics selection. Preliminary results of NLCR (= 10) showed that 76% of the positive blood cultures (22/29) fit this selection criterium. Using NLCR only 70 blood samples (54%) would have been tested with molecular diagnostics. Data will be presented of the other markers, which will potentially increase the selective power.

P083

Correlating *Chlamydia trachomatis* bacterial load to population origin and symptoms

J.A.M.C. Dirks[1], A.A.T.P. Brink[2], A.G.C.L. Speksnijder[3], P.F.G. Wolffs[2], C.J.P.A. Hoebe[1]
[1]*GGD Zuid Limburg, Sexual Health, Infectious Diseases and Environmental Health, Geleen,* [2]*Maastricht University Medical Center, Medical Microbiology, Maastricht,* [3]*GGD Amsterdam, Medical Microbiology Laboratory, Amsterdam*

Introduction: *Chlamydia trachomatis* (CT) is the most common bacterial sexually transmitted infection (STI) worldwide. The number of CT positive patients at STI clinics in the Netherlands has been found to increase annually, and accounted for approximately 15.000 new infections in 2012. When infected, at least 50% of men and 70% of women remain asymptomatic, hampering antibiotic treatment and potentially resulting in severe sequelae such as pelvic inflammatory disease, ectopic pregnancy and infertility. Almost all previous studies that have focused on correlating bacterial load to the presence of symptoms have looked at high-risk populations with contradicting results. The aim of this study was to compare the bacterial load in patients from a high risk cohort with patients from a population-based cohort and correlate this to the presence of symptoms.
Methods: A random selection of 1336 CT-positive men and women from a national population-based chlamydia screening (CSI) (n = 769; 207 men, 562 women) and attendees of the STI-clinic in South Limburg (n = 567; 216 men, 351 women) were systematically assessed for CT-load. Bacterial load was based on the copy number of major outer-membrane protein (MOMP) per copy number of eukaryotic cells (HLA) derived by real-time quantitative polymerase chain reaction (qPCR).
Results: The mean logarithmic bacterial load for male CSI-participants was 1.32 copies/HLA, while in STI-clinic visitors 1.09 copies/HLA were detected (p = 0.031). Contrastingly, in women a bacterial load of 1.45 copies/HLA was detected in the CSI-cohort, while 1.48 copies/HLA were detected in the STI-cohort (p = 0.54). When correlating bacterial load to symptoms, a total of 961 STI- and CSI-patients were grouped together for further analysis. An increase in bacterial load resulted in an increased risk for the presence of symptoms in men (OR 2.0 (1.14-2.01)) but not in women (OR 1.15 (0.91-1.46)). In this line, an increase in micturition frequency (OR 2.00 (1.12-3.55)) in men and painful micturition was found in both men (OR 1.74 (1.04-2.93)) and women (OR 1.33 (1.03-1.71)) to be associated with an increase in CT load.
Conclusion: This is the largest study of *C. trachomatis* load in both a population-based and a high-risk cohort to date. Our findings could not support the hypothesis that the bacterial *C. trachomatis* load is lower in the general population than in a high-risk population, instead we found the opposite in men. We found a correlation between bacterial load and the presence of urinary tract symptoms in both men and women, supporting previous reports on this topic.

P084

Cefotaxime resistant *Enterobacteriaceae* in fecal samples of horses

M. Leendertse[1], J. Hordijk[2], A. Timmerman[2], M.J.P. Theelen[2], T.J.P. Spoormakers[3], E. Broens[2], J.A. Wagenaar[2]
[1]*Academisch Medisch Centrum, Dept. of Medical Microbiology, Amsterdam,* [2]*Faculty of Veterinary Medicine, Utrecht University, Dep. of Infectious Diseases and Immunology, Utrecht,* [3]*Equine Clinic Lingehoeve Diergeneeskunde, Lienden*

Introduction: Concerns remain about the rapidly increasing prevalence of highly resistant micro-organisms in the environment and as a potential reservoir for infection in humans and animals. The epidemiology of these resistant micro-organisms is poorly understood, since data are lacking about the presence in most reservoirs and routes of transmission. Currently, many studies are performed on the prevalence of extended spectrum bèta-lactamase (ESBL) and plasmid mediated AmpC-producing (pAmpC) *Enterobacteriaceae* amongst different food-producing and companion animals. As people have close contact with dogs, cats and horses these animals may be a source for direct transmission of ESBLs to humans. In 2012, we showed that 40-50% of dogs and up to 20% of cats tested, carried ESBL-producing *Escherichia coli* in their feces. Recently, Dierikx et al. (JAC 2012) described 8% ceftiofur/cefquinome resistance among *Enterobacteriaceae* from clinical horse samples.
The aim of this study was to estimate the prevalence of carriage of ESBL- and pAmpC-producing *Enterobacteriaceae* in horses.
Methods: Fecal samples from horses, without gastrointestinal problems, were collected at two locations: 25 at the Clinic for Equine Medicine of the faculty of Veterinary

Medicine (Utrecht) and 26 at the Equine Clinic Lingehoeve Diergeneeskunde (Lienden). Most horses were submitted to the clinics because of orthopedic problems. Informed consent was obtained from all owners and a brief questionnaire on previous antibiotic usage and previous hospitalization of the horses was completed.
Each fecal sample was inoculated onto MacConkey agar, supplemented with 1 mg/L cefotaxime (McC+) and in Luria Bertani liquid broth, supplemented with 1 mg/L cefotaxime (LB+). After overnight incubation, the LB+ culture was plated onto McC+. Suspected colonies were biochemically tested, or tested by MALDI-TOF, for species determination. The cefotaxime-resistant *Enterobacteriaceae* were tested using the Check-Point CT101 micro-array, and were subsequently screened by sequence-analysis to identify ESBL/pAmpC genes and mutations in the chromosomal ampC promoter region.
Results: From the first set of 25 fecal samples, nine cefotaxime-resistant *E. coli* isolates were selected from six samples and further characterized. So far, three isolates were positive for CTX-M-group 1 genes and five isolates harbored mutations in the chromosomal AmpC promoter region, which may result in derepression of the AmpC-gene. All five were AmpC-type-18 variants. From the second set of 26 fecal samples, six cefotaxime-resistant *E. coli's* and one *Rahnella aquatilis* were isolated from six horses. These isolates are further tested, currently. Half of all isolates were obtained directly from the primary McC+ agar, the other half grew only after pre-incubation in LB+ broth and subsequent culture onto McC+ agar.
Conclusion: The preliminary data of this study demonstrate that 12 of 51 (23.5 %) horses harbor cefotaxime-resistant *Enterobacteriaceae*, which are predominantly *E. coli*.
In this study no correlation was found between the presence of cefotaxime resistance and the use of antibiotics, or previous hospitalization.
This study shows the potential role of horses in the complex epidemiology of ESBLs in different reservoirs.

P085

An outbreak of a multidrug-resistant *Acinetobacter baumannii* strain in a Dutch animal intensive care unit

M. Leendertse[1], J.A. Wagenaar[2], J. Hordijk[2], J.H. Robben[2], E. Broens[2]
[1]*Academisch Medisch Centrum, Dept. of Medical Microbiology, Amsterdam,* [2]*Faculty of Veterinary Medicine, Utrecht University, Utrecht*

Introduction: From June till September 2012 an outbreak of multidrug-resistant (MDR) *Acinetobacter baumannii* took place at the veterinary intensive care unit (ICU), Utrecht. It started with an intubated dog, as he developed respiratory failure after eating the baclofen of his owner. *A. baumannii* was isolated from the tip of the tube, yet there were no signs of infection. The second isolate was obtained from urine of a catheterized dog that was admitted with renal failure. He was treated with neomycin. The third isolate came from another catheterized dog with renal failure. As leptospirosis was in the DDx, he was admitted in isolation, adjacent to the ICU. He never entered the main ICU and was treated with amikacin. The last strain was isolated from a dog with a chronic wound that was treated with enrofloxacin because of a mycoplasma infection. Sporadic colonies of *A. baumannii* were cultured, no treatment was initiated.
At human ICUs, outbreaks of (MDR) *A. baumannii* are well known and hard to control. On animal ICUs, these outbreaks are rarely described.
Methods: Biochemical species identification was confirmed by sequence-analysis of the ribosomal polymerase-subunit (rpoB) gene. Broth microdilution and E-tests were performed. The presence of carbapenemases was tested using the Checkpoint CT102 micro-array and additional PCRs for blaOXA genes, this was followed by a PCR directed at the combination of the OXA-51-like gene cluster with the ISAba1 element. Genotyping was performed by pulsed field gel electrophoresis (PFGE), using the ApaI restriction enzyme. Twenty-five swabs were taken from the ICU (among other places boxes, waterpoints, pillows, ventilation machine, computer keyboards) and cultured using gentamicin-containing media.
Results: The strains were resistant to fluoroquinolones, third generation cephalosporins, kanamycin, tetracycline, trimethoprim-sulfamethoxazole and sensitive for colistin, amikacin and neomycin. Minimum inhibitory concentrations (MICs) for meropenem and imipenem were 1-1.5 g/ml and 2-3 g/ml (both S = 2/R > 8), respectively. The OXA-51-like- and ISAba-gene combination, which confers carbapenem resistance was present in all strains. PFGE demonstrated that the four strains were identical. No *A. baumannii* was cultured from the swabs taken at the ICU.
Conclusion: During the summer of 2012 a (as for today) self-limiting outbreak of a MDR *A. baumannii* strain took place at the veterinary ICU, Utrecht. Four dogs were cultured positive with indistinguishable MDR isolates of *A. baumannii*.
Despite the presence of the OXA-51-like- and ISAba1-gene combination, the MICs for meropenem and imipenem indicated (intermediate) susceptibility to these antimicrobials.
Although, no advanced cleaning action took place, the strain could not be cultured from the ICU, which was in parallel with the fact that no additional clinical samples were found positive for MDR *A. baumannii*.
We can only speculate on the source of this outbreak; the owner of the first dog that was found positive, is known to suffer from a diabetic foot (for which the baclofen). As for today we are trying to find out, if the owner is a known carrier of this *A. baumannii* strain.

P086

Hepatitis E virus infection in the Netherlands; diagnostic performance of two commercial enzyme-linked immunosorbent assays

N. Hilt, F.F. Stelma, M. van der Horst, J.P.H. Drenth
UMC St Radboud, Dept. of Medical Microbiology, Nijmegen

Introduction: *Hepatitis E* is enterically transmitted and infection usually results in a self-limited inflammatory disease of the liver. The HEV seroprevalence in the Dutch population is ~1,9%. Currently available commercial serological assays are validated for genotype 1 and 2 antigens, but most European human infections result from genotype 3. The diagnostic properties of the available assays for HEV genotype 3 are unclear. The aim of this study was to validate two commercial serological assays for the diagnosis of the HEV genotype present in the Netherlands.
Methods: We selected 2 panels for this validation study. The first panel consisted of 6 HEV PCR positive samples, 12 samples of assumed HEV negative from patients with no known pathology, and 20 samples from patients with a clinical diagnosis known to cause cross reactivity (rheumatoid factor latex plus particle agglutination assay positive n = 5, Lues n = 5, primo-infections EBV n = 5, and acute mycoplasma infections n = 5).
The second panel consisted of serum samples from a population with minimal HEV-exposure (children aged 12-24 months, n = 90). Both panels were tested with two HEV-specific IgG and IgM enzyme-linked immunosorbent assays: WANTAI (Wantai Enterprise Co., Beijing, China) and MICROGEN (Microgen GmbH, Neuried, Germany). The tests were performed according to the manufacturer's instructions.
Results: All HEV-positive samples were detected by WANTAI IgM and IgG ELISA. The WANTAI IgM was negative in 30 of 32 negative and cross reactive samples. The WANTAI IgG was negative in 23 of 32 negative and cross reactive samples. Only 5 out of 6 HEV-positive samples were IgM positive with the MICROGEN ELISA, while all 6 samples were positive with the IgG ELISA. The MICROGEN IgM and IgG were negative in 28/32 (negative) and 26/32 (crossreactive) samples. The WANTAI ELISA tested positive in 2/90 (IgG) and 0/82 (IgM) samples derived from the minimal exposure cohort. Sensitivity and specificity of the WANTAI IgM was higher than that of the MICROGEN IgM ELISA (100% versus 83% and 94% versus 88% respectively). However, the specificity of the MICROGEN IgG (81%) was higher than that of the WANTAI IgG ELISA (72%).
Conclusion: The diagnostic test properties of WANTAI ELISA appear to be better in the acute phase of HEV-infection. When testing for post exposure or seroprevalence, MICROGEN performs better. The high false positive rate observed in the IgG ELISAs in adults, but not children, suggests that the HEV-seroprevalence in Dutch adults may be underestimated.

P087

Species within the *Pseudomonas fluorescens* intrageneric cluster are erroneously identified as *Burkholderia pseudomallei* or *Francisella tularensis* by different commercial identification systems

M.J.C. van den Beld, D.W. Notermans, F.A.G. Reubsaet
RIVM, Center for Infectious diseases and screening, Bilthoven

Introduction: *Burkholderia pseudomallei* is a gram-negative bacterium that habitats the soil in tropical countries. It is also a CDC category B potential agent for bioterrorism and requires BSL-3 safety conditions to culture.
Bacterial strains from an industrial water circuit from the Netherlands were initially identified using API 20 NE as or possibly *B. pseudomallei*, and they were sent to the RIVM for confirmation. These strains were identified using a polyphasic approach as *Pseudomonas brenneri* and *Pseudomonas gessardii*.
Because a mis-identification as *B. pseudomallei* can have far-reaching consequences, further research into related strains was conducted. Identification with automated systems in most Dutch laboratory settings replaced identification with API 20 NE, therefore this study will focus on identification of the *P. brenneri* and *P. gessardii* related strains with VITEK 2 and matrix-assisted laser desorption ionization -time of flight (MALDI-TOF) mass spectrometry.
Methods: The sequence of the 16S rDNA genes from the mis-identified *P. brenneri* and *P. gessardii* strains were compared with all strains in our own 16S rDNA database. Strains with a 100 percent match and which were identified as or possibly *B. pseudomallei* using API 20 NE were selected. The typestrains of *P. brenneri* and *P. gessardii*, were purchased. This selection resulted in twenty-eight strains, of which two of them are of human origin. All of these strains were subjected to a polyphasic approach, which includes identification with API 20 NE, an extensive phenotypic identification, a 16S rDNA sequence analysis and a fatty acid analysis. The department Medical Microbiology and Immunology of the St. Antonius Hospital in Nieuwegein performed an identification using the VITEK-2 and a MALDI-TOF analysis.
Results: The API 20 NE score for all selected strains, but the typestrain of *P. gessardii*, confirmed a possibility of *B. pseudomallei*. With the polyphasic approach, all strains were identified as *P. brenneri* or *P. gessardii*. With

MALDI-TOF, 93% of the results are in concordance with the polyphasic approach. The two strains with a discrepancy are the two with a human origin; they were identified as *Pseudomonas proteolytica*. Using VITEK-2, the strains were determined as different species*; P. fluorescens, Pseudomonas aeruginosa, Pseudomonas pseudoalcaligenes, Comamonas testosteroni, Oligella ureolytica, Aeromonas salmonicida, Achromobacter denitrificans*, different species from the *Moraxella* group, but also *Francisella tularensis*. The latter species is listed as a CDC category A potential agent for bioterrorism, is highly pathogenic and requires BSL-3 safety conditions to culture.

Conclusion: In this study, the polyphasic approach is the gold standard. For a fast and high-through-put method, MALDI-TOF is the best choice. All tested identification systems based on biochemical characteristics, have poor results for identification of species within the *P. fluorescens* intrageneric cluster, probably due to the absence of references in their databases. API 20 NE scored a low discrimination' for *B. pseudomallei*, and all VITEK results had a low discrimination for the result *F. tularensis*.

Nevertheless, if such a score is obtained one must exclude the possibility of this organism because of the high impact in laboratory measurements and public health.

Po88

Detection and occurence of plasmid-mediated AmpC in highly resistant gram-negative rods

E.A. Reuland[1], J.P. Hays[2], D.M.C. de Jongh[2], E. Abdelrehim[1], M. van Keulen[1], I. Willemsen[3], J.A.J.W. Kluytmans[3], P.H.M. Savelkoul[1], C.M.J.E. Vandenbroucke-Grauls[1], N. Al Naiemi[4]

[1]*VU University Medical Center, Dept. of Medical Microbiology & Infection Control, Amsterdam,* [2]*Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases , Rotterdam,* [3]*Amphia Hospital, Medical Microbiology & Infection Control, Breda,* [4]*Laboratory for Medical Microbiology and Public Health, Enschede*

Introduction: Because of screening difficulties, plasmid-mediated AmpC (pAmpC) producing organisms often go undetected, even though pAmpC may actually contribute to resistance to bèta-lactam antibiotics. The aim of this study was to evaluate different methods for the detection of pAmpC among group I *Enterobacteriaceae* (*E. coli*, *Klebsiella* spp, *P. mirabilis*, *Shigella* spp and *Salmonella* spp). We also estimated the frequency of occurrence of pAmpC among group I highly resistant gram-negative rods (HR-GNRs) in the Netherlands. HR-GNRs are defined as those resistant to bèta-lactams and/or carbapenems, or alternatively at least resistant to both quinolones and aminoglycosides.

Methods: The strains used were 503 HR-GNRs group I, isolated from hospitalized patients in 2007 (Willemsen et al. Infect Control Hosp Epidemiol 2011) and 21 pAmpC positive isolates previously confirmed by PCR (Perez-Perez J Clin Microbiol, 2002). Screening for the presence of pAmpC was performed using the following criteria: reduced susceptibility to ceftazidime and/or cefotaxime (positive ESBL screening according to the Dutch national guideline), and/or reduced susceptibility to cefoxitin (EUCAST and CLSI). Two disk-based tests, with cloxacillin and boronic acid as inhibitors, and Etest with cefotetan-cefotetan/cloxacillin, were used for phenotypic confirmation. Multiplex PCR was used as gold standard.

Results: pAmpC was detected in 13 out of 503 group I isolates (2.6%) and comprised 9 CMY-2, 3 DHA-1, and 1 AAC-1. Of these isolates, 335 fulfilled the screening criteria and thus were available for evaluation. The test panel, including 21 previously determined pAmpC positive isolates showed a sensitivity of 100% and a specificity of 9% for reduced susceptibility to ceftazidime and/or cefotaxime as screening method; combining this with reduced susceptibility to cefoxitin yielded a sensitivity of 97% and a specificity of 90%. This latter in combination with the inhibitor-based combination disk diffusion test with cloxacillin yielded the best sensitivity (94%) as a phenotypic confirmation method with a specificity of 61%.

Conclusion: 1. For routine detection, a screening strategy based on reduced susceptibility to cefoxitin combined with a positive ESBL screening, and confirmation with a combination disk diffusion test with cloxacillin, appears most useful.

2. pAmpC was present in 2,6%. Due to its plasmidal location, however, further increase in the occurrence of this resistance mechanism can be expected.

Po89

Prevalence of community-acquired plasmid-mediated AmpC in the Netherlands

E.A. Reuland[1], T. Halaby[2], J.P. Hays[3], D.M.C. de Jongh[3], H.D.R. Snetselaar[1], P.J.M. Elders[1], P.H.M. Savelkoul[1], C.M.J.E. Vandenbroucke-Grauls[1], N. Al Naiemi[2]

[1]*VU University Medical Center, Medical Microbiology & Infection Control, Amsterdam,* [2]*Laboratory for Medical Microbiology and Public Health, Enschede,* [3]*Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam*

Introduction: The objective of this study was to determine the prevalence of plasmid-mediated AmpC (pAmpC) bèta-lactamases in community-acquired gram-negative bacteria in the Netherlands. There is currently very little information available regarding the prevalence of these bèta-lactamases in the Dutch community. Further, pAmpC could represent an important resistance threat if its prevalence is found to be similar to that of other circulating bèta-lactamases.

Methods: Community-acquired faecal samples, consecutively obtained between August 12 and December 13, 2011, were analysed. In total 550 samples were obtained from healthy volunteers approached via five general practices, affiliated to the Academic General Practice Network, VUmc. Screening for pAmpC was performed with selective enrichment broth and inoculation on a selective screening agar, aimed at detection of bèta-lactamases. Confirmation of AmpC-producing Enterobacteriaceae was performed with two double disc combination tests: cefotaxim and ceftazidim with either boronic acid or cloxacillin as inhibitor. Species identification and antibiotic susceptibility testing were performed using the Vitek-2 system (bioMrieux). Micro-array (Checkpoints) was also done for group I *Enterobacteriaceae* (*E. coli*, *Klebsiella* spp, *P. mirabilis*, *Shigella* spp and *Salmonella* spp). Multiplex PCR was used as gold standard for detecting pAmpC. 16S rRNA PCR and AFLP were performed as required, identification of plasmids was performed using PCR-based replicon typing.

Results: The mean age of the participants was 51 years (range: 18-91), 61% were females. pAmpC was present in 7 *E. coli* isolates (7/550, 1.3%; 95% CI 1.3-1.4): 6 CMY-2 and 1 DHA. ESBL-encoding genes were found in 52/550 (9.5%; 95% CI 8.8-10.3) isolates; these were predominantly blaCTX-M genes. Two strains had both ESBL genes (one CTX-M-1 and one CTX-M-15) and pAmpC. AFLP showed no genetic relatedness between the *E. coli* isolates. Among pAmpC-producing isolates IncI1 (5/7), ColE (5/7), ColEtp (3/7), FIB (3/7), Frep (2/7), R (1/7) and FIA (1/7) were identified.

Conclusion: 1. Our data show a prevalence (1.3%) of pAmpC in community-acquired isolates that cannot be neglected.

2. Importantly, most of the pAmpC producing isolates were actually determined to be ESBL negative. Therefore resistant isolates are increasing, however via an ESBL algorithm they will not be detected. These data point to a clinical relevance that might be underestimated.

Po90

Novel multiplex RT-PCR for the detection of lineage specific strains

I.M.J.G. Sanders[1], W. Knetsch[1], E. Claas[1], M. Wilcox[2], E.J. Kuijper[1], J. Corver[1]

[1]*LUMC, Dept. of Medical Microbiology, Leiden,* [2]*Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom*

Clostridium difficile, an anaerobic gut pathogen, has rapidly emerged as a leading cause of antibiotic associated diarrheal disease in the developed world. Since 2002, the rate and severity of hospital acquired *C. difficile* infections (CDI) increased coincident with the emergence of two problematic PCR ribotypes (RTs) 027 and 078. Rapid identification of problematic strains is essential for preventing the spread of these strains. Recently, our laboratory described two genetic markers that are present in the genomes of PCR RT027 and 078 strains and several other highly related PCR RT strains. Comparative genome analysis (multi-locus sequence typing on 7 housekeeping genes) confirmed that PCR ribotype strains sharing the same genetic marker belong to phylogenetically coherent lineages (< ie. lineage 2 and 5).

In the present study primers and probes were developed to target the genetic markers as well as the toxin B (tcdB) gene and the glutamate dehydrogenase (GDH) gene. All targets were amplified in one multiplex real time (RT) PCR reaction. The developed multiplex assay was validated on a large collection of clinical samples. In total, 526 diarrhea samples were prospectively collected and included in the study, of which 101 samples (19.2%) were positive in cytotoxigenic culture. The results were compared to the appropriate gold standard (toxigenic culture, culture and PCR ribotyping).

Compared to cytotoxigenic culture, sensitivity, specificity, positive predicted value (PPV), and negative predicted value (NPV) for the tcdB probe was 91%, 97%, 87%, and 98%. Compared to Culture, sensitivity, specificity, PPV, and NPV for the GDH probe was 83%, 97%, 87%, and 96%. Compared to PCR ribotyping (culture positive fecal samples only), sensitivity, specificity, PPV, and NPV for the 027 probe was 90%, 96%, 87% and 98% and for the 078 probe was 75%, 100%, 100% and 99%.

Our multiplex RT PCR assay is rapid and displays sufficient performance (sens, spec, PPV and NPV) to use it as a first screening test in an algorithm for diagnosing CDI. Simultaneously to diagnosing CDI, this multiplex assay is able to detect strains belonging to lineage 2 (PCR RT027) & lineage 5 (PCR RT078).

Po91

The influence of antibodies on the innate immune response to respiratory syncytial virus infection

M. Vissers, I. Schreurs, M.I. de Jonge, R. de Groot, G. Ferwerda

RUMC, Laboratory of Pediatric Infectious Diseases, Nijmegen

Respiratory syncytial virus (RSV) can cause severe bronchiolitis in young infants. Not all cases of severe RSV infection can be explained by the known risk factors and the pathogenesis of disease severity is poorly defined. Young infants are not able to produce their own antibodies; they depend on antibodies that are derived from the mother. Evidence suggests that these maternal antibodies might have an influence on severity of infection. This study tries to identify the influence of antibodies on the immune response to RSV infection.

Previous experiments have shown that antibodies (Ig's) present in human serum (HS) prevent RSV from infecting

cells. However, stimulation of human PBMCs with RSV in combination with HS induces a synergistic increase in interferon-γ (IFN-γ) production. Stimulation of cell subsets after monocyte depletion of PBMCs showed that monocytes alone did not show this synergistic increase in IFN-γ production. However, the monocyte depleted portion of the PBMC showed a strong synergistic response. This IFN-γ response was very fast and could be measured after 4 hours on mRNA level and after 24 hours on protein level, suggesting an unexpected but direct stimulation of e.g. T-cells or NK-cells. To study whether the synergistic increase in IFN-γ is dependent on immune complexes, other HS components or infectious versus non-infectious virus, we stimulated human PBMCs with RSV and BPL-inactivated RSV in combination with HS and Ig depleted HS. Measurements of cytokine levels suggest that antibodies are the main cause of this synergistic increase in IFN-γ production.
We performed a micro-array for transcriptome profiling on the previous experiment to examine which pathways are involved in this synergistic increase and we will also study whether NK-cells or T-cells are responsible for the increase in IFN-γ production.

P092

Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in an STI population: performances of Presto, Lightmix Kit, and COBAS Amplicor with urine specimens and urogenital swabs

S.P. Verweij, T.A. Schuurs, J.F.W. Weel, S. Ouburg, S.A. Morré
VU University medical center, Medical microbiology and infection control, Amsterdam

Introduction: Urogenital *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are the most prevalent bacterial sexually transmitted infections (STI) in the Netherlands. In western society, highly sensitive and specific DNA or RNA amplification tests to detect CT and NG, are commercially available, and have increased detection rates as compared to conventional techniques including culture. The COBAS Amplicor assay (Roche, CA, USA) has been the most widely used system for CT and NG detection, in the Netherlands. Newly developed dual detection systems for *C. trachomatis* and *N. gonorrhoeae* are implemented in Europe in the last 2 years including the Presto CT/NG assay (Goffin Molecular Technologies, Houten, the Netherlands) and the Lightmix Kit 480HT NG and CT (TIB MOLBIOL, Berlin, Germany). This study assessed the performances of the three aforementioned tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.
Methods: A sample size of 1128 clinical samples (561 urines and 567 urogenital swabs, from both men and women) was used. All tests were performed according to the protocols provided by the respective manufacturers. The results of the three tests were compared to an alloyed gold standard, defined as two or three tests positive per sample.
Results: The sensitivity, specificity, positive predicative value (PPV), and negative predictive value (NPV) for *C. trachomatis* detection in urine samples using Presto were 100,0%, 99,8%, 98,1%, 100,0%, respectively; for Lightmix Kit: 94,2%, 99,8%, 96,1%, 99,4%, respectively; for COBAS Amplicor: 92,3%, 99,6%, 96,0%, 99,2%, respectively. In urogenital swabs the sensitivity, specificity, PPV, and NPV for *C. trachomatis* for both Presto and COBAS Amplicor were 100,0%, 99,8%, 97,7%, 100,0%, respectively. For Lightmix Kit this was 100,0%, 99,6%, 97,7%, 100,0%, respectively. Calculations for *N. gonorrhoeae* could not be made due to a low prevalence.
Conclusion: All three assays had a high sensitivity, specificity, PPV, and NPV for *C. trachomatis*, with best overall performance for the Presto CT/NG assay.

P093

Carbapenemase detection by matrix-assisted laser desorpion ionization time-of-flight (MALDI-TOF) mass spectrometry in a routine diagnostic laboratory

A. Russcher[1], S. Paltansing[1], D.W. Notermans[2], E.J. Kuijper[1]
[1]*LUMC, Dept. of Medical Microbiology, Leiden,* [2]*Centre for Infectious Disease Control, Bilthoven*

Introduction: Carbapenemases hydrolyze carbapenem antibiotics, resulting in resistance to carbapenem antibiotics. They are classified according to their amino-acid homology as belonging to Ambler class A (e. g KPC), B (e.g. NDM, VIM) or D (e.g. OXA-48). Recent results in detection of hydrolysis by mass spectrometry seem promising. This study compares different methods to detect carbapenemase activity on a Microflex LT mass spectrometer published so far, and subsequently validates the assay on clinical isolates for use on a routine basis in a diagnostic laboratory.
Methods: Firstly, four methods to detect carbapenem hydrolysis were compared in terms of accuracy and practicality in 5 carbapenemase positive isolates (Ambler class A: 2; B: 1; D: 2) and 5 carbapenemase negative strains. Secondly, one of these methods was selected to validate this assay on 53 clinical isolates (Ambler class A: 10; B: 18; D: 25; confirmed by molecular analysis) from LUMC and RIVM and 15 carbapenemase negative strains. In short, methods differ mainly in pre-treatment of the samples before Microflex analysis and matrix solution:
Method A (Burckhardt et al, 2011): incubation 2 h in presence of ertapenem 0.5 mg/L in NaCl 0.45%; analysis with a-HCCA matrix.
Method B (Sparbier et al, 2011): incubation 3 h in presence of ertapenem 0.5 mg/L in 10 mM ammonium hydrogen citrate; analysis with a-HCCA matrix.
Method C (Sparbier et al, 2011): incubation 3 h in presence of meropenem 0.5 mg/L in 10 mM ammonium hydrogen citrate; analysis with a-HCCA matrix.
Method D (Hrabak et al, 2012): incubation 3 h in presence of 0.1 mM meropenem in 20 mM TrisHCl with 0.01% SDS; analysis with di-hydroxy benzoic acid.
Results: Method A, B and D identified carbapenem hydrolysis in 3/5 isolates correctly. Method C identified 4/5 isolates correctly; but misidentified one negative strain. Method B was further validated. 89% (25/28) of isolates of Ambler class A or B were correctly identified i.e. no intact ertapenem could be detected after incubation. In 2/3 isolates intact ertapenem was still present but intensity of peaks corresponding to ertapenem was reduced compared to the negative strains. All 15 negative strains were identified correctly. Ambler class D isolates however, were correctly identified in only 16% (4/25). In 16/21 isolates, again a reduced intensity of peaks corresponding to ertapenem was observed. Variation in duration of incubation or concentration of ertapenem could improve detection in Ambler class D isolates to a sensitivity of 67% (ertapenem 0.05 mg/L, 4 h incubation) with a specificity of 100%.
Conclusion: Although method C identified one more isolate correctly; method B was found to be more easily applicable and unequivocally interpreted. This study subsequently shows that carbapenem hydrolysis in clinically relevant isolates of Ambler class A or B can be reliably detected by mass spectrometry. Ambler class D isolates showed only partial hydrolysis and could therefore not be reliably detected. These isolates did show high MIC-values for carbapenems; suggesting that other resistance mechanisms contribute to carbapenem resistance. This also suggests that quantification of hydrolysis could be a valuable addition to the assay.

P094

Gene transcription profiling of MRSA ST398 during interaction with porcine nasal mucosa in an *ex vivo* colonization model

P. Tulinski[1], B. Duim[1], F. Wittink[2], J.P.M. van Putten[1], J.A. Wagenaar[1], A.C. Fluit[3]
[1]*Utrecht University, Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht,* [2]*University of Amsterdam, MicroArray Department,, Amsterdam,* [3]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht*

Introduction: *Staphylococcus aureus* is a common human and animal opportunistic pathogen. Nasal carriage of *S. aureus* is a risk factor for various infections. Methicillin-resistant *S. aureus* ST398 is highly prevalent in pigs in Europe and North America. The mechanism of successful pig colonization by MRSA ST398 is poorly understood. We developed a nasal colonization model of porcine nasal mucosa explants to identify molecular traits involved in nasal MRSA colonization of pigs. Here we report the analysis of the transcriptome of MRSA ST398 strain S0462 during colonization on the explant epithelium.
Methods: Mucosa explants were prepared by stripping the mucosal membrane from the surfaces of the ventral turbinates and septum from euthanized and exsanguinated pigs. One cm^2 explants were colonized with pig MRSA ST398 isolate S0462 using a standard adhesion assay (using a bacterial inoculum of $3*10^8$ CFU/ml). Colonization was assessed within 180 min after adhesion. At 0, 30, 60, 90, and 180 min bacteria were harvested from the epithelium layer and total RNA was isolated and used in a micro-array assay. Interaction of S0462 with pig epithelial cells was analyzed in a time course experiment with four replicas per time point. Expression values were calculated as fold changes with respect to t = 0. Transcripts that are significantly up- or down-regulated (adjusted p = 0.05) were visualized in a hierarchical clustering with a heatmap.
Results: During the 3 h of the colonization assays, a number of *S. aureus* transcripts showed upregulation, while others showed downregulation. Analysis of the nature of the mRNA transcripts showed significant changes in the transcription of genes encoding a two-component system, or involved in fatty acid biosynthesis, amino acid biosynthesis, and oxidative phosphorylation. Interestingly, a pathogenicity island, SaPIbov5, was only partly expressed (int, vwbp and some of the hypothetical proteins). Additionally, von Willebrand factor-binding protein (vwbp, located on SaPIbov5) was up-regulated in the first phase of colonization. The presence of this protein has been suggested to be indicative of a host adaptation mechanism for *S. aureus* for animals. Colonization did not result in significant changes in transcripts of main virulence associated genes or main colonization factors with the exception of the downregulation of capsular genes and genes involved in alpha-hemolysis and the up-regulation of genes encoding cysteine extracellular proteinase (scpA). To investigate the role of the vwbp and scpA genes in colonization, knockout mutants of these genes were constructed. Both mutants showed similar colonization levels as the parent strain.
Conclusion: We provide evidence of alterations of MRSA ST398 gene transcripts during interaction of *S. aureus* with porcine nasal epithelium. Changes mainly involved regulatory and metabolic genes, while transcript levels of virulence associated genes remained largely unchanged during colonization. Individual inactivation of two of the up-regulated genes (vwbp and scpA) did not alter the nasal *S. aureus* colonization. These results suggest that nasal colonization with MRSA ST398 is a complex event that

is accompanied with changes in bacterial regulation and metabolic adaptation.

P095

The shape of non-typeable *Haemophilus influenzae* prevents neutrophil-mediated killing

J.D. Langereis, P.W.M. Hermans
Radboud UMC Nijmegen, Laboratory of Pediatric Infectious Diseases, Nijmegen

Introduction: Non-typeable *Haemophilus influenzae* is a major pathogen causing exacerbations of chronic obstructive pulmonary disease and there is a clear relationship with non-typeable *Haemophilus influenzae* colonization of the lungs and disease severity. Also, non-typeable *Haemophilus influenzae* causes huge healthcare burden with almost 24 billion spend on chronic obstructive pulmonary disease every year in the USA alone. Usually, non-typeable *Haemophilus influenzae* colonizes the nasal mucosa without signs of inflammation but when non-typeable *Haemophilus influenzae* gets into the lung it causes acute inflammation. Neutrophils, which are immune cells equipped with various mechanisms to kill bacteria, are recruited to the lungs to clear the pathogen. However, non-typeable *Haemophilus influenzae* is not cleared effectively from the lungs of chronic obstructive pulmonary disease patients.
The objective of this study was to identify non-typeable *Haemophilus influenzae* genes essential for growth and survival in the presence of neutrophils.
Methods: We isolated peripheral neutrophils from whole blood of healthy donors in all our experiments. A random non-typeable *Haemophilus influenzae* strain 3655 transposon mutant library with ~25.000 independent mutants was generated. The mutant library was incubated for 3 hours in the absence or presence of neutrophils with an MOI of 1 in the presence of 10% heat-inactivated pooled human serum. We used the next generation sequencing Tn-seq technology to identify genes essential for non-typeable *Haemophilus influenzae* strain 3655 in the presence of neutrophils and opsonizing antibodies.
Results: Non-typeable *Haemophilus influenzae* strain 3655 growth was not decreased in the presence of freshly isolated neutrophils with an MOI of 0.1, 1, 10 and 100, which confirmed previous published data (Juneau, Infect Immun., 2011). We identified that transposon mutations in 18 non-typeable *Haemophilus influenzae* strain 3655 genes showed decreased survival or growth in the presence of neutrophils compared to the control condition without neutrophils. Among others, we identified genes involved in the bacterial shape, showing that the bacterial shape has a very important role in this resistance to neutrophils-mediated killing. Gene deletion of rod shape-determining protein (CGSHi3655_04250) decreased survival in the presence of neutrophils compared to the wild-type bacterium, which confirmed the Tn-seq results.
Conclusion: The shape of non-typeable *Haemophilus influenzae* might affects survival in the presence of neutrophils. The mechanism is currently under investigation.

P096

Correlation in complement resistance and IgM binding between genetically identical non-typeable *Haemophilus influenzae* isolates collected from the nasopharynx and middle ear fluid of children with otitis media

J.D. Langereis[1], T.M.A. van Dongen[2], K. Stol[1], R.P. Venekamp[2], A.G.M. Schilder[2], P.W.M. Hermans[1]
[1]*Radboud UMC Nijmegen, Laboratory of pediatric infectious diseases, Nijmegen,* [2]*Julius Center for Health Sciences and Primary Care, UMC Utrecht, Dept. of Epidemiology, Utrecht*

Introduction: Non-typeable *Haemophilus influenzae* is often found to be present in the middle ear of young children with otitis media. In general, non-typeable *Haemophilus influenzae* is efficiently killed by complement-mediated killing. Initiation of classical complement activation is largely dependent on IgG and IgM binding to the bacterial surface, as well as binding of CRP to phosphorylcholine present in the lipooligosaccharide structure of non-typeable *Haemophilus influenzae*. We have previously found that strains collected from the middle ear of children with otitis media exhibit increased complement resistance. It is however not known if complement resistance increases in the middle ear, or if it is already present in the nasopharynx. The objective of this study was therefore to investigate whether there is a correlation in complement resistance of genetically identical non-typeable *Haemophilus influenzae* isolates from the middle ear and the nasopharynx of children with otitis media.
Methods: Non-typeable *Haemophilus influenzae* was simultaneously isolated from the nasopharynx and middle ear fluid of 62 children with recurrent acute otitis media, chronic otitis media with effusion or children with acute tympanostomy tube otorrhea. Multi-locus sequence type, complement resistance, IgG binding, IgM, binding and phosphorylcholine expression was determined.
Results: In 41 children (67%), genetically identical non-typeable *Haemophilus influenzae* pairs were identified in the middle ear and the nasopharynx. There was no statistically significant difference in complement resistance between the genetically identical strains collected from the nasopharynx and the middle ear. Isolates collected from the middle ear showed a high correlation for complement resistance and IgM binding with the genetically identical non-typeable *Haemophilus influenzae* isolates from the nasopharynx, whereas this was not the case for IgG binding and phosphorylcholine incorporation into lipooligosaccharide.
Conclusion: Complement resistance and IgM binding of genetically identical non-typeable *Haemophilus influenzae* isolates collected from the middle ear and nasopharynx of children with otitis media were highly correlated. This finding might indicate that complement resistance does not change when present in the nasopharynx or the middle ears of children with otitis media.

P097

Non-typeable *Haemophilus influenzae* biofilm formation is not correlated to phosphorylcholine incorporation into the lipooligosaccharide structure of clinical isolates

S. Marti[1], C. Puig[1], P.W.M. Hermans[2], J. Liñares[1] J.D. Langereis[2]
[1]*Hospital Universitari de Bellvitge-Universitat de Barcelona, IDIBELL, Barcelona, Spain,* [2]*Radboud UMC Nijmegen, Nijmegen*

Introduction: Non-typeable *Haemophilus influenzae* is a gram-negative, human-restricted pathogen. This bacterium usually colonizes the nasopharynx asymptomatically, but is also one of the major pathogens causing otitis media in children, and pneumonia and exacerbation of chronic obstructive pulmonary disease in the elderly.
Non-typeable *Haemophilus influenzae* can be found in biofilms, either attached to the epithelium or free floating in the middle ear fluid. One of the factors found to induce non-typeable *Haemophilus influenzae* biofilm formation is the incorporation of phosphorylcholine into the lipooligosaccharide structure of the bacterium (Hong, J Bacteriol, 2007).
In this study, we determined phosphorylcholine expression and biofilm formation of non-typeable strains isolated from the oropharynx of healthy children, sputum from patients with chronic obstructive pulmonary disease and from sputum of patients with community acquired pneumoniae.
Methods: Non-typeable *Haemophilus influenzae* strains were isolated from oropharyngeal samples of 30 healthy children, from sputum of 27 patients with stable (n = 8) or during an exacerbation (n = 19) of chronic obstructive pulmonary disease and from sputum of 30 patients with community acquired pneumonia. Biofilm formation was determined in a static biofilm formation assay. Phosphorylcholine expression was determined with monoclonal antibody TEPC-15 and visualized with a FITC-coupled secondary antibody by flow cytometry.
Results: Non-typeable *Haemophilus influenzae* isolates collected from healthy children, patients with chronic obstructive pulmonary disease or community acquired pneumoniae presented large variation in biofilm forming capacity, but showed no statistical significant differences in biofilm formation between the different groups. Unexpectedly, biofilm formation negatively correlated with the presence of phosphorylcholine in the lipooligosaccharide structure of non-typeable *Haemophilus influenzae*. Furthermore, we show an increased phosphorylcholine expression for non-typeable *Haemophilus influenzae* grown in a biofilm compared to planktonic growth, which corroborates previous published data (West-Barnette, Infect Immun., 2006).
Conclusion: Although increased expression of phosphorylcholine for selected non-typeable *Haemophilus influenzae* was shown to increase biofilm formation (Hong, J Bacteriol, 2007), expression of phosphorylcholine is not correlated to biofilm formation in a large collection of clinical non-typeable *Haemophilus influenzae* isolates collected from different patient groups.

P098

Epstein-Barr virus large tegument protein contributes to innate immune evasion through interference with Toll-like receptor signaling

M. van Gent[1], S. Braem[1], A. de Jong[2], N. Delagic[3], J. Peeters[1], G.J. Brak-Boer[1], P.N. Moynagh[3], E. Kremmer[4], E.J.H. Wiertz[1], H. Ovaa[2], B. Griffin[1], M.E. Ressing[1]
[1]*UMC Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*The Netherlands Cancer Institute, Division of Cell Biology, Amsterdam,* [3]*National University of Ireland Maynooth, Dept. of Biology, Maynooth, Ireland,* [4]*Institute of Molecular Immunology, Helmholtz Zentrum München, München, Germany*

The human herpesvirus Epstein-Barr virus (EBV) is a large, enveloped DNA virus carried by more than 90% of the adult world population. EBV is the causative agent of infectious mononucleosis and is associated with several malignant tumors. After primary infection, EBV persists for life in latently-infected B cells expressing only a limited set of viral proteins. For the production of new infectious virus, EBV reactivates from these B cells and over 90 viral gene products are expressed during this lytic phase.
EBV can activate members of the Toll-like receptor (TLR) family. TLRs are innate pattern recognition receptors with an important role in (initial) detection of (viral) infections. Upon ligand binding, TLRs initiate direct antiviral defense mechanisms and shape the ensuing adaptive immune response. To withstand host immunity, EBV has acquired sophisticated immune evasion strategies. The aim of this study is to determine if and how EBV counteracts innate immune activation signaled through TLR.
We have found that EBV interferes with expression and/or activation of several TLRs during latent as well as lytic infection. To identify the viral gene products responsible,

we studied the effects of individual EBV proteins on TLR signaling. TLR signaling pathways are subject to regulation by cellular protein ubiquitination, a process controlled by ubiquitin ligases and deubiquitinases. EBV does not appear to encode ubiquitin ligases. We, therefore, focused on three EBV-encoded proteins reported to exert *in vitro* deubiquitinase (DUB) activity. Using active site-directed probes, we show that one of these – a conserved herpesvirus large tegument protein – is a functional DUB expressed during the late phase of lytic EBV infection. The N-terminal part of this large tegument protein contains the catalytic site for DUB activity and inhibits TLR-mediated activation of NF-κB at or downstream of the TRAF6 signaling intermediate. A catalytically inactive mutant of this EBV protein did not reduce NF-κB activation, indicating that DUB activity is essential for blocking TLR signal transduction.
In conclusion, the EBV large tegument protein obstructs TLR-mediated innate immunity. Interference with pattern recognition receptors capable of sensing EBV provides a mechanism by which this virus can obstruct host antiviral responses.

P099

Delayed diagnosis and therapy of infant botulism

M.B. Haeseker[1], V. Hira[1], D. van Waardenburg[1], C. Swaan[2], K. Heijman[3], F. van Zijderveld[4], E. Stobberingh[1]
[1]MUMC, Dept. of Medical Microbiology, Maastricht, [2]Centre for Infectious Disease Control, National institute for Public Health, RIVM, Bilthoven, [3]Dept. of Sexual Health and Infectious Diseases and Environmental Health, Public Health Services South Limburg, Geleen, [4]Central Veterinary Institute Wageningen, Lelystad

Introduction: Infant botulism is rare in Europe, possibly due to the recommendation that honey, a known source of spores of *Clostridium botulinum*, should not be given to infants less than one year. Diagnosis and therapy can be easily delayed. Here, we describe the case of a patient with infant botulism, who was initially treated for bacterial sepsis.
Case description: A two months old baby presented with difficulty in swallowing, dyspnoe and irritability at the emergency department of a peripheral hospital. During a sepsis work-up a cardiac arrest occurred. After successful reanimation and intubation the patient was transferred to the paediatric intensive care of the Maastricht University Medical Centre. Amoxicillin-clavulanic acid (and one dose of gentamicin) was started on the suspicion of sepsis. All bacterial cultures, i.e. blood, faeces and sputum, and molecular tests, i.e. respiratory viruses and enterovirus were negative. CRP was stable between 25-60 mg/L. Antibiotics were stopped on day 10. To assess possible brain damage after reanimation, an MRI cerebrum and EEG were made. The MRI was normal and the EEG showed multiple focal abnormalities, consistent with post-anoxic encephalopathy. After an attempt to detubate, physical examination showed a mimicless infant with severe hypotonia, and the patient was re-intubated. The clinical diagnosis of infant botulism was suspected on day 21 and a detailed history from the parents revealed that honey was used during breastfeeding. The electromyography showed low CMAP (Compound Muscle Action Potential) on stimulation, consistent with infant botulism. Botulinum serotype A toxins were detected in serum and in very high concentrations in the faeces with mouse bioassay on day 24. *Clostridium botulinum* was cultured from the faeces. The patient received human botulinum immunoglobulins (BabyBIG) on day 30, slowly recovered and was successfully detubated on day 48.
Conclusion: This case report shows that the diagnosis and therapy of infant botulism is challenging and it demonstrates that despite preventive messages for parents, infant botulism still occurs in the Netherlands. The most sensitive means of botulism detection is the mouse bioassay. Antibiotics are controversial, as more toxins are released. Equine botulinum antitoxin is available in most countries; human botulinum immunoglobulins are expensive and only available in the US. Antitoxin stops further accumulation of toxins in the nerve terminals and shortens the clinical course. Antitoxin should be started as soon as possible. Supportive care is essential; this includes artificial feeding and often intubation and ventilator support. Clinicians, especially general practitioners, paediatricians, neurologists and clinical microbiologists should always think of infant botulism when an infant less than one year presents with sepsis-like symptoms, especially when the patient is not improving on antibiotics. A detailed history with specific attention for honey consumption is very helpful.

P100

Creating a human resistome; from metagenomics to antimicrobial resistance

B.B. Wintermans
VU University Medical Center, Clinical Microbiology and Infection Control, Amsterdam

Introduction: Detection of antimicrobial resistance is crucial for infection control and antibiotic choice. Current phenotypic detection methods just expose the tip of the iceberg, identifying specifically targeted genes in single cultured bacterial strains. Better understanding of antimicrobial resistance may be achieved by using genotypic detection methods, e.g. by next-generation sequencing.
In this study we demonstrate how to create a total 'human resistome' by identifying resistance genes in metagenomic data.
Methods: The Human Microbiome Project published metagenomic datasets of stool samples by using Next-generation sequencing. These samples were collected from healthy participants who did not receive antibiotics in the prior 6 months. In this study custom software was made to efficiently organise and search through 25 of these datasets. These datasets were screened against a library of 587 known antimicrobial resistance genes. With these data a 'human resistome' could be constructed per participant.
Results: In total 15 of the 587 genes present in our library were found at least once among the participants. These genes were; ccrA, cfxA, cepA, cfiA, ermF, tet-A, tet-M, tet-X, cat, cmlA1, fexA, ACT-9, LEN, IMP-12 and aadA8b. In the majority of the samples genes were found that are common in *Bacteroides* spp. (cfxA, ermF) and genes that represent tetracycline resistance (tet-M, tet-X). Sporadically chloramphenicol resistance genes (cat, cmlA1, fexA) were found. Genes that can be found in *Enterobacteriaceae* were obtained incidentally (LEN, ACT-09, IMP-12, aadA8b). There was a large diversity in (relative) abundance of these genes in the resistome of the 25 participants.
Conclusion: We demonstrate that it is feasible to create a resistome from metagenomic data and that metagenomic data can be used to test for presence of specific resistance genes. This creates opportunities to use metagenomics for epidemiology and for clinical diagnostics. Other antimicrobial resistance genes in less abundant intestinal flora, e.g. *Enterobacteriaceae*, can probably be found by increasing sequence depth.

P101

Transcriptional response of *S. pneumoniae* to co-infection with influenza A virus

M.N. Habets, H.J. Bootsma, A. Zomer, P.W.M. Hermans, D.A. Diavatopoulos
Radboud University Nijmegen Medical Centre, Laboratory of Pediatric Infectious Diseases, Nijmegen

Combined infections with influenza A virus (IAV) and the bacterium *Streptococcus pneumoniae* (the pneumococcus) are responsible for a significant proportion of all infection-related deaths worldwide. The IAV pandemics of the 20th century (1918, 1957 and 1968) clearly demonstrated that infection with IAV facilitates the progression of *S. pneumoniae* from a commensal organism to a potentially fatal pathogen. In all three pandemics the majority of individuals are thought to have succumbed to a secondary bacterial infection, predominately caused by the pneumococcus. A large number of different mechanisms have been hold accountable for this exacerbation of disease such as modulation of neutrophil function by IAV and alterations in innate signaling and cytokine/chemokine responses. Whilst co-infection with IAV has been shown to result in increased bacterial adherence, replication and invasion, the transcriptional and or metabolic changes in the pneumococcus during this process remain largely unknown. Here, we examine the transcriptional response of *S. pneumoniae* to co-infection with influenza A virus.
Human epithelial A549 cells were infected with influenza A virus or mock for 24 h *S. pneumoniae* was then added to the cells for 2 h at a multiplicity of infection of 10. RNA was isolated from both attached and non-attached bacteria and the transcriptome was subsequently analysed using pan-pneumococcal expression arrays. Our results suggested that pneumococci adhering to virus-infected cells had a similar gene expression profile compared to mock infected cells. Unexpectedly, we found that non-adherent bacteria downregulated the expression of the glutamine biosynthesis operon (glnRAPQ) in the presence of IAV. Interestingly, the gln operon was also downregulated in co-infection with respiratory syncytial virus (RSV), suggesting that this might be a general phenomenon in respiratory viral-pneumococcal co-infections. Glutamine synthesis is important in nitrogen metabolism and our laboratory has previously shown that this operon plays a role in pneumococcal adhesion and virulence. It is therefore tempting to speculate that viral co-infection induces environmental changes that lead to detachment, potentially via glnRAPQ. This may result in increased transmission to other hosts or migration to other mucosal sites such as the lungs. Our current focus is to study the *in vivo* pneumococcal response to IAV co-infection in a mouse model of infection, in order to be able to identify changes in specific pathways that could help to better understand and diagnose the transition from asymptomatic colonization to the development of disease.

P102

Taxonomic composition of the distal gut microbiota in inflammatory bowel disease (IBD) patients with changing disease activity over time

J. Penders, M. Pierik, E.S. Wills, D. Jonkers
Maastricht University Medical Centre+, Dept. of Medical Microbiology, Maastricht

Objectives: A role of the indigenous microbiota in the development of exacerbations in IBD is supported by studies showing that diversion of the fecal stream and antibiotic treatment can reduce disease activity.
Although differences have been reported between active and inactive IBD patients, limited studies have investigated the microbiota in relation to changes in disease course over time. The aim of our study was to prospectively study the fecal microbiota in IBD patients developing an exacerbation.
Methods: The study was conducted within the context of a prospective follow-up cohort of IBD outpatients. Fecal samples from 10 Crohn's disease (CD) and 9 ulcerative

colitis (UC) patients during remission and subsequent exacerbation were included. Active disease was determined by colonoscopy, fecal calprotectine > 150 g/g or 5-fold increases versus remission. Exclusion criteria were antibiotic use, pregnancy, daily enemas, and medication changes between consecutive samples. The microbial taxonomic composition was assessed by 454 pyrosequencing of the 16S rRNA V1-V3 regions.

Results: After quality control, 6,194 - 11,030 sequences per sample were available for subsequent analyses. Patient-specific shifts in bacterial diversity and taxonomic composition were observed during exacerbation compared to remission, but overarching shifts within UC or CD were not observed. However, changes in the bacterial community composition between remission and exacerbation as assessed by Bray-Curtis dissimilarity, were significantly larger in CD compared to UC patients (0.59 vs. 0.42, respectively; p = 0.025). Disease location had a significant effect on microbial community membership as determined by unweighted UniFrac-based principal coordinate analysis (PCoA) in UC (p = 0.013).

Thiopurine use was found to be a significant cause of clustering as shown by PCoA of all patients. Additionally, thiopurine use was associated with a decreases in bacterial richness (Chao1 501.2 vs. 847.6 in non-users; p < 0.001) and diversity (Shannon index: 5.13 vs. 6.78, respectively; p < 0.01).

Conclusion: Shifts in microbial composition during exacerbations seem to be patient-specific, which is more pronounced in CD than in UC patients. To identify more general patterns related to disease or patient characteristics, a larger number of study subjects is required. Furthermore, thiopurine decreases microbial diversity and affects microbial composition and should be considered when studying the intestinal microbiota in relation to disease course.

P104

Comparison of urine samples and penile swabs in the detection of human papillomavirus in men using the SPF10 Line Probe Assay

F.M.H.P.A Koene[1], P.F.G. Wolffs[1], A.A.T.P. Brink[1], W.G.V. Quint[2], C.A. Bruggeman[1], C.J.P.A. Hoebe[3]

[1]MUMC+, Medical Microbiology, Maastricht, [2]DDL Diagnostic Laboratory, Rijswijk, [3]South Limburg Public Health Service, Sexual Health, Heerlen

Background: Penile swab sampling is the gold standard when testing for human papilloma virus (HPV) in men. Urine sampling could provide an easier to use sampling material. Therefore we compared the detection of a broad spectrum of HPV types in urine samples and penile swabs in HIV-negative men in order to see if urine is comparable to the penile swabs in the detection of HPV by a highly sensitive detection method.

Methods: First void urine and self-obtained penile swab samples were collected from 120 men visiting a Sexually Transmitted Infections (STI) clinic in South Limburg, the Netherlands. When exclusion criteria were met, the results of 111 men, with a mean age of 29,4 years, were analyzed. Broad-spectrum HPV DNA amplification and mucosal HPV genotyping were performed using the SPF_{10}-DEIA-$LiPA_{25}$ system (SPF_{10} HPV LiPA, version 1; manufactured by Labo Bio-Medical Products, Rijswijk, the Netherlands). The DNA was extracted with Qiagen DNA mini kit and spiked with an internal control plasmid. The HPV types detectable by SPF_{10} $LiPA_{25}$ are types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 (high risk HPV (hrHPV)), 34, 53, 66, 68/73/97, 70 (possible hrHPV) and 6, 11, 40, 42, 43, 44, 54 and 74 (low risk (lrHPV)). The possible high risk types in this study are considered to be high risk types.

Results: In total in 75 (67.6%) of 111 men HPV DNA was found using the SPF_{10} $LiPA_{25}$ system. In 73 men HPV DNA was detected in the penile swab or both samples and in two men HPV DNA was reproducibly detected only in the urine sample. Sixty-six paired samples were concordant in being HPV positive or negative. Of these samples 30 (27%) pairs were positive and 36 (32%) pairs were negative for HPV. Eleven of these 30 matching samples were concordant in genotype and fourteen pairs were comparable (= 1 genotype identical). The remaining five pairs were discordant (none identical) in their genotypes. Interestingly three of these discordant and one comparable urine sample contained high risk types (types 16, 39 en 66) which were not found in the swab. Furthermore, the two positive urines with a negative swab contained high risk types as well (types 53 and 66 respectively).

Conclusion: Urine samples are not comparable to penile swabs in the detection of HPV in men. This could possibly be explained by a low viral load in urine, despite the highly sensitive detection method. Besides the low viral load, a difference in affinity of HPV-types with epithelial cells in the urethra versus the epithelial cells of the glans could be an important factor as well. Notably, in the urine sample of six men, (possible) high risk types were found that could not be detected in the penile swab alone. The clinical relevance of this finding needs to be further investigated, but this finding could be an indication of the additional value of the urine sample next to the penile swab in the detection of HPV in men.

P105

Veterinarians' perceptions on veterinary antimicrobial usage and reduction. A qualitative study

D.C. Speksnijder[1], A.D.C. Jaarsma[2], A.C. van der Gugten[3], T.H.J.M. Verheij[3], J.A. Wagenaar[1]

[1]Faculty of Veterinary Medicine, Utrecht University, Infectious Diseases & Immunology, Utrecht, [2]Center for Evidence-Based Education, Academic Medical Centre, UvA, Amsterdam, [3]Julius Center for Health Sciences and Primary Care, Utrecht Medical Centre, Utrecht

Introduction: Veterinary antimicrobial usage might pose a risk for public health. As antimicrobial usage in Dutch livestock is relatively high compared to other European countries, there is a strong need for reduction. Antimicrobials for veterinary use are available only by prescription of veterinarians. The aim of this study was to identify factors influencing prescription behavior and possible targets for interventions aiming at reducing veterinary antimicrobial prescription.

Methods: Semi-structured interviews were conducted amongst eleven veterinarians primarily working with different livestock species (pigs, poultry, veal, dairy). An interview guide was developed based on literature dealing with prescription behavior of medical doctors and conversations with veterinary experts. The interviews were recorded, transcribed and analyzed.

Results:

Veterinarians attitude

Respondents consider it their moral and professional duty to treat sick animals. High veterinary antimicrobial usage is seen as a consequence of current livestock husbandry systems in which animals are easily affected by diseases. Prophylactic antimicrobial usage to control endemic animal diseases is therefore by some considered more prudent than curative usage accompanied with animal welfare problems. Some respondents consider high veterinary antimicrobial usage a real threat for public health while others consider other causes of human antimicrobial resistance more important than veterinary antimicrobial usage.

Farmers' role

Economic considerations play an important role. Farmers often see antimicrobial treatment as more profitable on short term than investing in expensive measures to prevent animal diseases. Other farmers are perceived tolack the motivation or skills to actively improve animal health. Veterinarians cannot force farmers to implement specific preventive measures. Because of their financial dependency from their clients, veterinarians benefit from a stable relationship with farmers and don't easily refuse to prescribe when requested for antimicrobials by a farmer. A strong need is felt to establish a more independent position of veterinarians.

Paradigm shift

In order to reduce veterinary antimicrobial usage, emphasis should be on intensifying veterinary herd health consultancy services (VHHCS) with the aim to keep animals healthy with a maximal production. However, some obstacles can be identified. Offering VHHCS differs from the classical curative task of veterinarians and requires new skills like identifying risk factors for animal diseases, proper communication skills to transfer specific recommendations to farmers and knowledge of how animal nutrition, genetics and housing conditions contributes to animal health. However, according to the respondents, many veterinarians still don't master these competences well enough to provide optimal VHHCS whereby animal health will be improved with a minimal usage of antimicrobials. The paradigm shift from generating most of the income from curative veterinary practice including drugs selling towards having most income from consultancies to prevent diseases is not yet generally accepted by farmers. They might hesitate to hire VHHCS for they are not convinced of the beneficial (financial) effects of it.

Conclusions: Veterinary antimicrobial usage is often related to financial and management choices of farmers. Advisory and communication skills of veterinarians need improvements. Solutions should be found to minimize the conflicting dependency relation of veterinarians to their farmers and the dependency on veterinary drug dispensing

P106

Development of a rapid multiplex PCR assay for accurate diagnosis of neonatal sepsis

M. van den Brand[1], A.R. Rubenjan[1], R.P.H. Peters[2], A.C. Catsburg[3], F. Broeke[1], M. Remm[4], T. Kõressaar[4], F.A.M. van den Dungen[1], M.M. van Weissenbruch[1], P.H.M. Savelkoul[1], M.P. Bos[3]

[1]VUmc, Medische Microbiologie en Infectiepreventie, Amsterdam, [2]Anova health institute, Tzaneen, South Africa, [3]Microbiome, Houten, [4]University of Tartu, Dept. of Bioinformatics, Tartu, Estonia

Introduction: Blood culture (BC) is the gold standard for diagnosis of neonatal sepsis, but diagnostic impact is negatively affected by considerable time to results and a suboptimal sensitivity which is partly caused by the small inoculation volume. Clearly, faster diagnostic tools are needed as this condition may be life threatening. Efforts to improve the diagnostic standard have focussed around broad range PCR assays which are qualitative and require additional processing steps for species identification. Therefore, we aimed to develop a quantitative multiplex PCR assay for rapid diagnosis of the most prevalent bacterial pathogens of neonatal sepsis to be used directly on blood samples.

Methods: Three multiplex real-time PCR assays using hydrolysis probes were designed to detect coagulase negative staphylococci (CNS), *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa* and *Serratia marcescens*. Specific PCR targets were selected from the literature or designed using genome comparison bioin-

formatic tools. Analytical sensitivity and specificity were assessed for each PCR. For specificity testing a panel of > 40 bacteria and fungi was selected. Sensitivity was also determined using spiked blood samples from healthy volunteers.
Performance of the multiplex PCR was compared to that of the monoplexes. The novel multiplex assay was clinically evaluated with neonatal whole blood samples, taken simultaneously with BC samples. A volume of 0.2 ml whole blood was used for bacterial DNA extraction with the Easymag automated system and amplified using a Lightcycler 480.
Results: New PCR targets were identified for identification of *Klebsiella* spp, *S. agalactiae, P. aeruginosa and S. marcescens* that showed 100% specificity, in silico and in PCRs. Target species were detected with a limit of detection ranging between 1 and 10 cfu/PCR except for *E. coli*. Comparison of the multiplex assay with the monoplexes with dilution series showed an equal sensitivity (e.g. median Cp difference 0.33 for *Klebsiella* spp.). In 7 examined clinical samples with growth in the BC (*S. aureus* or CNS) the novel assay could identify the pathogen correctly.
Conclusion: 1. We established a real-time multiplex PCR assay with high analytical sensitivity and specificity for diagnosis of bacterial pathogens in neonatal sepsis.
2. Preliminary clinical evaluation showed 100% concordance between the result of the PCR and blood culture.
3. This novel assay could possibly accelerate diagnosis.

P107

Probing the antibiotic resistance gene reservoir in the microbiota of a hospitalized patient by functional metagenomics and metagenomic shotgun sequencing

E.B. Bülow[1], T.B.G. Bello Gonzales[2], E.A.N.O. Oostdijk[1], L.O. Ogilvie[3], E.O. Oosterink[1], D.V. Versluis[2], M.W.J.P. van Passel[2], H.S. Schmidt[2], M.B. de Been[1], B.V. Jones[4], M.J.M.B. Bonten[1], R.J.L. Willems[1], W.V. van Schaik[1]
[1]*UMC Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*Wageningen University, Laboratory of Microbiology, Wageningen,* [3]*School of Pharmacy and Biomolecular Sciences, University of Brighton, Center for Biomedical and Health Science, Brighton, United Kingdom,* [4]*University of Brighton, School of Pharmacy and Biomolecular Sciences, Brighton, United Kingdom*

Introduction: To study the effects of hospitalization on the antibiotic resistance gene (ARG) reservoir (the resistome') of the human microbiota, conventional clinical culturing methods are insufficient. Metagenomic techniques that allow either the screening of environmental DNA pools for the presence of ARGs (functional metagenomics) or shotgun sequencing of metagenomic DNA will result in a more comprehensive description of the resistome. The resistome carried by the gut microbiota is particularly relevant in hospitalized patients, which are frequently at risk for infections with antibiotic-resistant bacteria. Here we aimed to determine the dynamics of the resistome in a patient during intensive care unit (ICU) hospitalization, subsequent transfer to another hospital ward and 9 months after hospital discharge. The patient received continuous antibiotic prophylaxis (selective digestive tract decontamination; SDD) during ICU stay. SDD helps to prevent infections by opportunistic pathogens. Whether SDD selects for antibiotic resistance among the gut microbiota is a subject of controversy.
Methods: To monitor the antibiotic resistance gene reservoir in this patient we applied both functional metagenomics and metagenomic shotgun sequencing.
Five fosmid libraries (from 0.8 Gbp to 2.6 Gbp in size) were constructed from fecal samples collected throughout hospitalization and after hospital discharge. The libraries were plated on LB with different antibiotics to identify resistant clones. *In vitro* transposon mutagenesis was employed to identify resistance determinants. Quantitative PCR (qPCR) assays were developed to quantify copy numbers of resistance genes in the total DNA pool. Selected fosmids were sequenced by Illumina sequencing. Metagenomic shotgun sequencing (> 5 Gbp sequence data/sample) was used to monitor the total resistome of the patient. Comparison of the obtained sequence data with an ARG database enabled us to describe the patient's resistome throughout hospital stay and upon discharge.
Results: Our functional metagenomics results show selection for antibiotic resistance in the gut microbiota during ICU stay and antibiotic prophylaxis, particularly among anaerobic commensals. The ARG that confers resistance to tobramycin (which is part of the SDD regimen) was strongly selected for during ICU stay of this patient.
Analysis of the genetic context of the cloned ARGs indicates that the ARGs may be mobilizable as they are contained on a DNA fragment that includes genes which are predicted to be involved in conjugation and recombination.
Further qPCR analysis showed that the identified tobramycin resistance gene is significantly more abundant in the gut microbiota of ICU patients than in the gut microbiota of healthy subjects, suggesting that SDD selects for resistance to tobramycin. Metagenomic shotgun sequencing allowed a more comprehensive overview of the resistome than functional metagenomics and identified a wide variety of resistance genes.
Conclusion: Hospitalization has a major effect on the reservoir of antibiotic resistance genes in this patient. The ARGs are not harbored by nosocomial pathogens but rather by anaerobic gut commensals. SDD appears to select for tobramycin resistance among this group of bacteria. Finally, we suggest that metagenomic shotgun sequencing presents a potential future monitoring tool to detect and quantify ARGs in the microbiota of hospitalized patients.

P108

Cowpox virus protein CPXV012 inhibits antigen presentation by blocking the MHC class I peptide loading complex

R.D. Luteijn
UMCU, Dept. of Medical Microbiology, Utrecht

Viral antigen presentation by MHC class I molecules on the cell surface is one of the principal mechanisms to trigger antiviral immune responses by patrolling T-cells.
To evade T-cell recognition, viruses have evolved different mechanisms that tamper with MHC class I antigen presentation. A principle strategy is blocking peptide loading onto MHC class I molecules by targeting components of the peptide loading complex (PLC), including the ATP-driven Transporter Associated with Antigen Processing (TAP), and Tapasin. The PLC mediates transport of proteasome-derived peptides over the ER membrane and subsequent loading onto MHC class I molecules. Inhibition of the PLC retains MHC class I in the ER and thus blocks presentation of viral antigens on the cell surface.
Recently, it was shown that cowpox virus (CPXV), a large dsDNA virus, also tampers with MHC class I antigen presentation. The viral proteins CPXV012 and CPXV203 are responsible for this effect. The 9kDa protein CPXV012 inhibits peptide transport into the ER lumen by interfering with TAP function through an undefined mechanism.
In this study, we show that CPXV012 binds specifically to the PLC, but does not disrupt the protein composition of the complex. Tapasin is dispensable for stability and functioning of CPXV012, however, TAP is required for stable expression of the viral protein. Peptide binding to TAP is not altered in the presence of CPXV012, but ATP binding is greatly reduced.
In conclusion, these results show that CPXV012 specifically targets TAP and blocks its ATP-binding domains, thereby withholding the energy necessary to drive the transport of peptides into the ER. CPXV012 is the first viral protein reported outside the herpesvirus family that targets TAP to avoid T-cell-mediated antiviral immune responses.

P109

Mono penicillins for all pneumococcal bacteraemia patients?

A.J.H. Cremers[1], J.F. Meis[2], G. Walraven[1], T. Sprong[2], P.W.M. Hermans[1], J.G. Ferwerda[1]
[1]*Radboud University Medical Centre, Paediatrics, Nijmegen,* [2]*Canisius-Wilhelmina Ziekenhuis, Medical Microbiology and Infectious Dise, Nijmegen*

Introduction: According to the Dutch antibiotics guidelines, preferred treatment for pneumococcal infections is monotherapy with penicillins (MP). Clear disadvantages of other treatment regiments are disturbances in the patient's natural flora and the development of antibiotic resistance. Still, these guidelines are followed hesitantly. In this study, we investigated whether a switch from empiric therapy to MP after blood culture positivity (BCP) is associated with mortality, while taking age, comorbidities and severity of disease into account.
Methods: Between January 2000 and June 2011, adults who were admitted to two Dutch hospitals with a bacteraemic pneumococcal infection (pneumonia, meningitis or unknown focus of infection) without treatment limitations were retrospectively included in the study. Detailed clinical data reflecting severity and outcome of disease were obtained in addition to data on antibiotic treatment.
Results: Three hundred and thirty-two patients were included in the study. Two hundred and seventy-nine patients suffered from pneumonia, 27 had meningitis and 26 had an unknown focus of infection. Two hundred and sixty-six patients were initially treated with antibiotics other than MP, of which 127 (47.7%) had a switch to MP after BCP (50.9%, 50.0% and 10.0% in pneumonia, meningitis and unknown focus of infection respectively). Mortality was lower in patients who had a switch to MP after BCP (3/127, 2.4%) compared with patients who did not (22/135, 16.3%) ($p < 0.0001$), while age, comorbidities and presence of sepsis at admission did not differ between the two groups. The mortality rate for pneumonia cases was 1.8% (2/113) in patients who had a switch to MP after BCP compared with 9.4% (10/106) in patients who did not ($p = 0.016$), while the distribution of the cases over the pneumonia severity index (PSI) classes and presence of empyema did not differ between the two groups. Within this study, the absence of switching to MP after BCP was associated with patients suffering from chronic obstructive pulmonary disease (COPD) ($p = 0.041$), although no association was observed with age, other comorbidities, presence of sepsis or empyema, distribution over PSI classes, admission to an intensive care unit or mechanical ventilation. Among COPD patients, mortality was lower in patients for who antibiotic treatment was switched to MP (0/27, 0%) compared with no switch to MP (8/44, 18.2%) ($p = 0.021$).
Conclusion: Given equal age, comorbidities and severity of disease at admission, a switch to MP after BCP in pneumococcal bacteraemia patients was associated with reduced mortality, particularly in pneumonia patients.

P110

Differentiation of *Citrobacter* species with and without chromosomally encoded AmpC enzymes by matrix-assisted laser desorption ionization time of flight mass spectrometry

R.J. Rentenaar[1], L.B.J. van der Velden[2], A.S. Jong[2], D. Vegt[2], S. Erkens-Hulshof[3], P.D.J. Sturm[3]

[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]UMC St Radboud, Nijmegen, [3]Laboratory for Pathology and Medical Microbiology, Dept. of Medical Microbiology, Veldhoven

Introduction: *Citrobacter freundii, C. braakii, C. youngae, C. murliniae and C. werkmanii* contain chromosomally encoded AmpC bèta-lactamase genes, resulting in (inducible) resistance against third generation cephalosporins associated with treatment failures. Rule 9.2 of the EUCAST expert rules states that in *Citrobacter freundii*, if measured susceptible *in vitro* to cefotaxime, ceftriaxone or ceftazidime, then, either monotherapy with any of these drugs should be discouraged, or, susceptibility testing results for these agents should not be reported to the clinicians. In contrast, *C. koseri, C. amalonaticus, C. farmeri, C. sedlakii, C. rodentium,* and *C. gillenii* lack chromosomally encoded ampC bèta-lactamases and susceptibility to bèta-lactam drugs can be reported as measured.
Since MALDI-TOF MS identifications are typically available before routine susceptibility results, the species identification and the accompanying intrinsic antimicrobial resistance can support the choice of antimicrobial treatment in addition to local epidemiology. We investigated whether the MALDI-TOF MS can reliably discriminate *Citrobacter* species with and without chromosomally encoded AmpC bèta-lactamases.
Materials and methods: 39 Stored *Citrobacter* isolates were thawed and subjected to MALDI-TOF MS analysis according to manufacturers' instructions (Bruker, Bremen, Germany). The reference identification method was 16S rRNA gene sequence analysis. AmpC bèta-lactamase was demonstrated by resistance to cefoxitin and/or inducible resistance to cefotaxim and/or ceftazidim in the disk approximation test using cefoxitin and imipenem as inducing agents.
Results: Based on the 16S rRNA sequencing, 23 isolates were *Citrobacter* species with chromosomal AmpC, and 16 isolates were species without chromosomal AmpC. MALDI-TOF MS correctly identified the 23 isolates as *Citrobacter* species with chromosomal AmpC. AmpC activity was phenotypically confirmed in all 23. Of the 16 *Citrobacter* isolates without chromosomal AmpC, 15 were correctly identified by MALDI-TOF MS. One isolate (*C. koseri*) was misidentified as *C. braakii*. One correctly identified *C. koseri* isolate displayed cefoxitin resistance which may be due to porin changes as neither inducible nor stable cefotaxim or ceftazidime resistance was demonstrated.
Conclusion: MALDI-TOF MS reliably discerns *Citrobacter* isolates with chromosomally encoded AmpC bèta-lactamases from those without. Thereby MALDI-TOF MS may aid in fast, identification based, treatment modifications in compliance with EUCAST expert rule 9.2. and may prevent inappropriate use of more broad spectrum antimicrobials in patients with infections with *Citrobacter* species not harbouring chromosomal AmpC bèta-lactamase.

P111

Mice are more susceptible to early than to late *Staphylococcus aureus* infections in the experimental biomaterial-associated infection model

M. Riool[1], V. Jaspers[2], L. de Boer[2], C.M. van der Loos[2], S.A.J. Zaat[1]

[1]AMC / University of Amsterdam, Dept. of Medical Microbiology, Amsterdam, [2]AMC, Dept. of Medical Microbiology, Amsterdam

Introduction: Infection of inserted or implanted medical devices ('biomaterials') can have disastrous consequences, including removal of the device. Implantation of a biomaterial provokes an inflammatory response known as the 'foreign body response'. *Staphylococcus epidermidis* and *Staphylococcus aureus* are the major causes of biomaterial associated infection (BAI), which in absence of a foreign body hardly ever cause infection. Formation of biofilms on the biomaterial surface is generally considered the main reason for these persistent infections, but *Staphylococcus epidermidis* has been shown to survive inside macrophages around biomaterials implanted in mice (Boelens et al. 2000), and was retrieved from peri-catheter tissue in humans (Broekhuizen et al. 2008). Biofilms or persistent infections are thought be the reason for late infections of the biomaterial. To study the pathogenesis of early (i.e. infection during surgery) and late biomaterial-associated infections, we compared an injection of *S. aureus* along the implanted biomaterial directly upon implantation of a titanium biomaterial to an injection of *S. aureus* 2 days after implantation of the biomaterial.
Methods: The inoculum was either injected directly along the implanted titanium biomaterial (i.e. early infection), or injected 2 days after implantation of the biomaterial (i.e. late infection). *S. aureus* ATCC 49230 was used in a concentration of $1x10^6$ CFU in 25 l. Mice were sacrificed at 1 and 4 days after implantation, when biopsies were collected to assess the bacterial colonization of both the biomaterial and the surrounding tissue. Each group consisted of 9 mice, each with 2 implants (n = 8). Multiple spectral imaging (MSI) was performed to stain macrophages, neutrophils and bacteria in one single section.
Results: After 1 and 4 days, we cultured significantly more bacteria from tissue with an early infection, than from tissue with a late infection ($p < 0.05$). All tissue biopsies of both types of infection were culture positive after 1 day, with less variation in the late infection group, suggesting that 2 days after implantation the host defense system is better capable to control the infection. This resulted in a significant reduction ($p < 0.05$) of culture positive tissue samples after 4 days in the late infection group (39%) compared to the early infection group (83%).
The titanium biomaterial showed a significant reduction in bacterial colonization after 1 day for the late infection, both in numbers of bacteria ($p < 0.001$) and the amount of culture positive samples ($p < 0.01$). After 4 days, no difference was observed on colonization of the biomaterial between early and late infection.
We characterized and compared the cellular immune response in single microscopic slides of mouse tissue with early and late infection, using the MSI technique.
Conclusion: The infection susceptibility of mice with an early infection is higher than of mice with a late infection.

P112

Properdin, PTX3, SAA and CRP predict disease severity in children with acute respiratory viral tract infections

I.M.L. Ahout[1], H.K. Brand[2], A. Zomer[2], H.W. Rutjes-van den Hurk[3], G. Schilders[3], M.L. Brouwer[4], C. Neeleman[5], A. Warris[2], P.W.M. Hermans[2], R. Groot de[1], G. Ferwerda[2]

[1]Radboud MC, Kindergeneeskunde, LKI, Nijmegen, [2]Radboud University Medical Centre, Dept. of Pediatrics, Nijmegen, [3]Hycult Biotech, Uden, [4]Canisius Wilhelmina Ziekenhuis, Pediatrics, Nijmegen,[5]Radboud University Medical Centre, Nijmegen, Dept. of Intensive Care Medicine, Nijmegen

Introduction: Respiratory viruses causing lower respiratory tract infections are a major cause for hospital admissions in children. Viral LRTI can deteriorate within hours or days into respiratory failure. Currently, It is not possible to predict the course of disease. Therefore many children are admitted for observation. Research on patients with bacterial infections showed associations between disease severity and levels of the plasma proteins C-reactive protein, PTX3, SAA, SAP and properdin. We evaluated whether these plasma levels could be used as markers to predict disease severity in children with viral respiratory infections.
Material and methods: Blood and nasopharyngeal washings from children < 3 years of age with clinical signs of a lower respiratory viral infection were collected within 24 h (acute) and after 4-6 weeks (recovery). Based on the level of supportive care patients were allocated into a mild (none), moderate (supplemental oxygen and/or nasogastric feeding) or severe (mechanically ventilation) group. Protein levels in plasma measured by ELISA were used in a linear regression analysis to design a prediction rule. This rule was subsequently tested in a new validation cohort.
Results: 104 children (52% male) were included. All markers, except properdin, differentiated between acute disease and recovery. All markers, except PTX3, showed significant differences in concentrations between at least two of the severity groups. A combination of CRP, SAA, PTX3 and properdin predicts severe disease better than any of the individual makers with 69% sensitivity and 90% specificity. Validation in a new cohort of 141 patients resulted in 71% sensitivity, 87% specificity, NPV 64% and PPV of 90%.
The prediction rule was not able to identify patients with a mild course of disease.
Conclusion: PTX3, SAA, CRP and properdin have additive value in the prediction of severe disease in viral lower respiratory tract infections. A combination of CRP, SAA, PTX3 and properdin had the strongest predictive capacity. Validation of this rule showed reproducible results.

P113

Combinatorial approaches for the creation and production of novel active lantibiotics: engineering of the protease NisP

M. Manuel, O.P. Kuipers

University of Groningen, Molecular Genetics, Groningen

Lantibiotics are a group of antimicrobial compounds that have in common the presence of (methyl)lanthionine and modified amino acids: dehydroalanine, dehydrobutyrine, D-alanine, halogenated amino acids, hydroxylated proline and many more. Some lantibiotics show activity in a range comparable to conventional antibiotics and are active against multidrug-resistant bacteria. These characteristics make them a very interesting group for their use in therapeutics. Lantibiotics are ribosomally produced as prepeptides that are modified by enzymes which often exhibit a high substrate tolerance.
In this project we will design a library of ~10,000 genes enconding for combinations of lanthionine rings fused to the leader peptide of nisin. The expression of the synthetic genes will be achieved by the nisin inducible system and the modification machinery of nisin: NisB (dehydratase), NisC (cyclase), and NisT (transporter). The presence of the leader peptide of nisin precludes a self-killing of the producer strain but leader peptide removal after production is mandatory to perform a high-throughput screening (e.g. microalginate beads) for the antimicrobial activity. Thus, an appropriate protease that can release the active lantibiotic is a main goal for the success of the project.
We have tested different proteases in prenisin: thrombin, factor Xa, endoprotease Glu-C and NisP. The presence of a mutated cleavage site at the end of nisin leader peptide can reduce the production level of the prepeptide but it is still possible to cut the leader peptide and get active molecules. Moreover, we have engineered a soluble tagged

NisP and we have shown that it can cleave prenisin with NisP, thrombin or factor Xa cleavage sequences correctly. In a small test, we have also shown the ability of NisP to activate *in vitro* and *in vivo* lantibiotic sequences different from nisin.

P114

Analysis of exposures and characteristics of cases of non-livestock associated CC398 MRSA in Denmark

L.B. van Alphen, J. Larsen, A. Petersen, A.R. Larsen, R.L. Skov

Statens Serum Institute, Dept. of Microbiological Surveillance and Infectioncontrol, Copenhagen, Denmark

In Denmark, the number of CC398 methicillin-resistant *Staphylococcus aureus* (MRSA) cases has increased in recent years, from 14 in 2007 to 164 in 2011. In most of these cases, exposure to live pigs is the most likely source of MRSA. However, the number of cases with no exposure to livestock is increasing. The increase in non-livestock associated (non-LA) CC398 MRSA in Denmark has led to the hypothesis that these isolates might be more easily transferred from person to person. In this study we investigated possible sources of infection and analysed whether non-LA isolates show different characteristics from LA isolates.

A questionnaire regarding possible exposures to CC398 MRSA were developed and sent out to the 33 out of 37 patients which did not report contact to livestock in the notification in 2010 and 2011 (4 patients were deceased at the start of the study). Isolates of cases without exposure to livestock were analysed by Pulsefield gel electroforesis (PFGE) using Cfr9I and genotypically characterised using PCR to identify spa-type and the presence of the genes tetM, tetK, scn, SCC mec 1A and luxF-luxS and compared to CC398 MRSA isolates from cases of LA-MRSA within the same geographical region and time of infection.

In total 23 questionnaires (72%) were returned. Two cases (9%) had exposure to livestock (pigs) and 3 cases were household contacts of other CC398 MRSA cases. Of the remaining 18 cases, two patients had contact to humans with livestock exposure. The majority of the 18 cases lived in close proximity to areas with intensive pig farming. PFGE analysis showed several PFGE subtypes with no clear pattern for non-LA CC398 cases. PCR showed a similar proportion of spa-type t034 (72%) among cases and controls and did not show an increase in the presence of genomic virulence determinants like scn, a human gene implicated to play a role in person to person transmission, or antibiotic resistance genes in the non-LA isolates.

The increase in CC398 MRSA cases without livestock association raises the question whether such isolates indeed are independent of livestock exposure and whether they might have different characteristics then LA CC398 MRSA isolates. Apart from close geographical location to pig farms we could not identify common epidemiological features among the non-LA cases. Isolates from these cases did not share a specific PFGE subtype or a PFGE subtype that is linked to a certain geographical location. Furthermore, there was no difference in presence of the studied genotypical characteristics of these isolates compared to LA CC398 MRSA isolates. An increase in non-LA isolates with increased person to person transmission could have severe consequences for the spread of MRSA. In this study, no difference in the presence of scn, which could contribute to an increase in person to person transmission were observed. However, an increase in person to person transmission dependent on other genetic elements is possible. Therefore non-LA cases should be closely monitored and this study will be extended with the non-LA cases from 2012.

P115

Successful delayed culture of *Neisseria gonorrhoeae* after storage using the ESwab™ system for up to 48 hours

C.M. Wind[1], H.J.C. de Vries[2], A.P. van Dam[3]

[1]*Municipal Health Service Amsterdam, STI Outpatient Clinic, Amsterdam,* [2]*Academic Medical Center, University of Amsterdam, Dermatology, Amsterdam,* [3]*Onze Lieve Vrouwe Gasthuis general hospital, Dept. of Medical Microbiology, Amsterdam*

Introduction: Recent reports on the spread of antimicrobial resistance (AMR) of *Neisseria gonorrhoeae* (Ng) like extended-spectrum cephalosporin resistant strains, have renewed the debate on the preferred diagnostic methods. Most guidelines recommend nucleic acid amplification tests (NAAT), which have higher sensitivity than direct culture. However, NAAT lacks the possibility to determine AMR. Routine diagnostic procedures ideally consist of a combination of NAAT and cultivation of Ng. To make such an approach efficient and practical for e.g. the general practitioners' setting, ideally cultures should only be implemented from samples with positive NAAT results. This requires a medium that allows Ng to survive until NAAT results are available. We evaluated the viability of Ng stored using the ESwab system.

Methods: We selected patients visiting the STI outpatient clinic at the Municipal Health Service (GGD) Amsterdam, with purulent discharge suggesting Ng infection. Urethral, cervical and/or anal swabs were collected as indicated, and plated directly on selective GC-lect agar plates. To evaluate the ESwab system, an extra Nylon Flocked swab for urogenital collection was obtained and stored in Liquid Amies ESwab medium (483CE, Copan Italia).

After storage for 1 hour at room temperature (RT) 200 l of the liquid medium was inoculated on selective GC-lect agar plates. Half (400 l) of the remaining medium was stored at RT, while the other half was stored at 4 °C. After storage for 24 and 48 hours 200 l samples of both stored liquid media were inoculated on GC-lect agar plates. All cultures were incubated at 37 °C and 5% CO_2 conditions for 72 hours. To evaluate the culture yield with the ESwab system, we used the direct plated selective GC-lect agar plates as gold standard.

Results: In December 2012, 53 ESwab samples were obtained: 33 urethral (UT), 7 cervical (CX) and 13 anal (AN). After storage for 1 hour at RT 34 (64%) ESwab system samples resulted in a positive Ng culture (UT: 25, CX: 1, AN: 8). This was fully consistent with results from direct plated selective GC-lect agar plates.

Storage of the ESwab system samples for 24 hours at 4 °C or at RT resulted in 32 (94%) successful Ng cultures (UT: 24, CX: 1, AN: 7). Storage for 48 hours at RT yielded only 13 (38%) viable Ng cultures (UT: 10, CX: 0, AN: 3), while storage at 4 °C for 48 hours yielded 25 (74%) viable Ng cultures (UT: 18, CX: 1, AN: 6).

Conclusion: Viable Ng cultures can be obtained from the ESwab system after storage at RT or 4 °C for 24 hours in 94% of cases and even after storage for 48 hours at 4 °C, in 74% of cases.

The ESwab system can be used for delayed Ng cultivation, which allows for preselecting NAAT positive samples.

Storing samples for Ng cultivation with the ESwab system could make Ng AMR monitoring more efficient and feasible for general practitioners.

P116

Effect of feeding type on the composition of the nasopharyngeal microbiota in infants

G. Biesbroek[1], A.T.M. Bosch[1], J.F. Keijser[2], X. Wang[1], E.J.M. van Gils[1], R.H. Veenhoven[3], A.M. Sanders[1], D. Bogaert[1]

[1]*UMC Utrecht, Dept. of Pediatric Infectious Diseases and Immunology, Utrecht,* [2]*TNO Earth, Environmental and Life Sciences, Research Group Microbiology and Systems, Zeist,* [3]*Spaarne Hospital, Research Center Linnaeus Institute, Hoofddorp*

Background and aims: Breastfeeding has shown to reduce episodes of respiratory infections in infancy. Several compounds in breastfeeding might contribute to this protective effect, nevertheless the exact mechanisms are unclear. Breastfeeding has shown to induce a more beneficial microbiome profile of the gut. We therefore hypothesized that breastfeeding might influence respiratory health by influencing bacterial colonization patterns, i.e. the microbiota, of the upper airways. We therefore compared the microbiota of the nasopharyngeal region of breastfed with formula-fed children.

Methods: From a well-characterized Dutch collection, nasopharyngeal swabs from 87 exclusively breastfed (until 6 months of age) and 101 formula-fed infants collected at the age of 6 weeks and 6 months were analyzed. The microbiota was determined by barcoded pyrosequencing of the V5-V7 hypervariable region of the small-subunit ribosomal RNA gene. Sequences were processed using the Mothur pipeline and operational taxonomic units defined on a 97% homology of sequences (OTU). Bacterial load was analysed by a universal Real-Time PCR of the 16S-rRNA gene.

Results: We observed age-related effects on microbiota composition with higher microbial diversity and differences in bacterial composition at 6 weeks compared to 6 months of age. Nasopharyngeal microbiota profiles of breastfed children were significantly different from formula-fed children (nMDS, p = 0.001) with significant increased abundance of clusters of *Corynebacterium* spp (RR = 7.3, p = 0.001) and *Dolosigranulum* (RR 14.9, p = 0.003) and decreased abundance and/or presence of a large cluster of Streptococcal species and anaerobes like *Prevotella* and *Veillonella*. In 6-month-old infants, this difference had merely disappeared.

Conclusion: Our data strongly suggest an effect of breast-feeding on bacterial colonization of the upper respiratory tract in 6-week-old infants. Observed differences in microbial profile may contribute to the protective effect of breastfeeding on respiratory infections in early infancy.

Acknowledgements

NWO-VENI grant 91610121 and ZonMw grant 91209010.

P117

Reproducible sampling of the intestinal microbiota in a clinical setting with rectal swabs

A.E. Budding, M.E. Grasman, A.A. van Bodegraven, P.H.M. Savelkoul

VU University medical center, Dept. of Medical Microbiology & Infection control, Amsterdam

Introduction: Recently, the gut microbiota has become an interesting potential target for clinical diagnostics. Currently, the most commonly used sample types for its analysis are feces and mucosal biopsies. However, as sampling method, storage and processing of samples have been shown to impact microbiota analysis, these sample types have limitations in respectively standardization and accessibility. An ideal sample type should be easily obtained in a standardized fashion with no preceding perturbation of the microbiota. Rectal swabs satisfy these criteria, but little is known about microbiota analysis on these sample types. In this study we investigate the applicability of rectal swabs for gut microbiota profiling in a clinical routine setting. We analysed optimal storage and processing of rectal swabs for clinical routine, reproducibility of profiles from rectal swabs and similarity to microbial profiles from fecal and mucosal samples.

Methods: Rectal swabs, mucosal biopsies, mucosal washings and fecal samples from 38 subjects were prospectively collected and analyzed by IS-pro, a high-throughput molecular fingerprinting method. Two rectal swabs were stored in RTF buffer at room temperature for two hours before freezing at -20 °C and one was immediately snap frozen. These samples were used to evaluate reproducibility of rectal swabs and effect of storage at room temperature. IS-profiles from rectal swabs were further compared to mucosal and fecal samples. All data analysis was performed with in-house developed software tools in combination with the Spotfire software package (TIBCO, Palo Alto, USA).
Results: IS-profiles from the two rectal swabs stored in RTF buffer at room temperature were highly similar (estimated correlation coefficients of IS-profiles > 90%) and were equally similar to the snap frozen rectal swab. Correlation of rectal swabs to feces was low (estimated correlation coefficients of IS-profiles 40-60%) and correlations to mucosal samples were slightly higher (50-70%). Correlations of fecal samples to mucosal samples were low (40-60%).
Conclusion: We find that rectal swabs give highly reproducible microbiota profiles that resemble mucosal adherent microbiota more closely than feces. Storage of swabs in RTF buffer of up to two hours at room temperature does not impact the results of subsequent microbiota analysis, making reproducible routine sampling in a clinical setting feasible.

P118

A standardized tool for site specific drug delivery using virus-like particles

C.H. van der Ark, M. van Veen, J.M. Bosman, W. Elings, H. de Vries, R. Nitschel, L. Sonnenberg, Y. Han, M. Vlot, T. Slijkhuis, J. Koehorst, A. Bhushan, N. Brouwers, M.W.J. van Passel, F. Hugenholtz
Wageningen University, Systems and Synthetic Biology, Wageningen

The international Genetically Engineered Machine (iGEM) competition is a competition for undergraduate students in synthetic biology. The Wageningen UR 2012 team entered the competition with the goal to improve site specific drug delivery. Medicines are generally active in a non-site-specific fashion, affecting the whole patient including healthy tissue. We attempt to target medicines specifically to diseased areas by packaging them inside virus-like particles (VLPs). VLPs are non-infectious viral capsids built solely from viral coat proteins. During the project, we used cowpea chlorotic mottle virus and Hepatitis B core antigen VLPs for production and isolated several variants of the turnip yellows virus coat protein gene. We designed a modular plug and apply system, based on complementarily charged peptide residues, that enables modifications to these coat proteins. The system facilitates the linkage of numerous ligands to the VLP, thereby creating site-specific carriers. Medicines can be packed using the plug and apply system or simply by addition during VLP assembly. We validated the production and assembly of both native as well as modified VLPs using electron microscopy and dynamic light scattering (DLS). DLS provides a novel way to quickly detect the presence of VLPs. In this work, (1) a carrier system was designed to decrease medicinal side effects for patients during treatment, that allows customization to a variety of diseases. A human body model was used to predict the applicability of the system. (2) This flow model of the human body confirmed that medicinal VLPs can indeed be used as universal carriers for site-specific drug delivery to reduce side effects. (3) Both modified and native VLPs have been shown to self-assemble *in vitro* after expression of the coat proteins in *E. coli*.

P119

Transmission by not isolated MRSA carriers still an issue despite good search & destroy

W.S.N. Lekkerkerk, M.J.A. Uljee, J.A. Severin, M.C. Vos
Erasmus MC, MMIZ, Rotterdam

Introduction: To keep a low prevalence of MRSA in the Netherlands, active search and destroy (S&D) policy and prudent use of antibiotic use are practiced. After two decades of S&D epidemiology changed. Two groups of MRSA may be recognized: MRSA with in the WIP guideline described risk factors (MRSA of known origin (MKO)), and MRSA without described risk factors (MRSA of unknown origin (MUO)). Patients without known risk factors are not screened and pre-emptive measures are not taken, allowing MRSA transmission in the hospital and thus outbreaks. We evaluated the results of S&D policy in a 1200-bed tertiary hospital between 2005 and 2010 and analzed the risk factors for transmission.
Methods: Data were retrospectively collected from standardized report forms. Primary cases (MUO or MKO) were defined as MRSA carriers found through active screening or clinical cultures, but not due to contact tracing. Index cases were cases whose detection of MRSA led to contact tracing. Secondary cases were found through contact tracing in- or outside the hospital, including patients and household members. Not isolated cases causing transmission were compared to those not transmitting to elucidate risk factors for transmission. Data were analyzed with SAS EG4.2 (descriptive analysis, Fisher's exact test with two by two tables and multiple regression models).
Results: In six years we identified 359 MRSA carriers, of which 229 primary cases (110 MUO and 119 MKO), 129 secondary cases (contact tracing; MKO) and 1 case with no data. The number of primary cases rose gradually over the years from 32 to 42 cases/year, with MUO consisting 50% of the total. Of the MUO cases, 87/110 (79%) were not isolated. For MKO this was 32/119 (27%) and 45/129 (35%) respectively for primary and secondary cases. Of all not isolated cases, 15/87 (17%) MUO index cases (2 on outpatient wards, 13 on inpatient wards) and 4/77 (5%) MKO index cases (secondary; 1 on an outpatient ward, and 3 on inpatient wards) caused transmission. After a not isolated stay of a MRSA carrier on an outpatient ward, 3% (3/104) resulted in transmission detection, while this was 15% (16/108) on inpatient wards. Furthermore, MUO carriers not isolated and causing transmission, had a mean admission stay of 14.6 days in comparison to 6.7 days when MUO did not transmit. Risk factors for transmission were a low Barthel-index, MUO, antibiotic use and artificial device (drain, intravascular line) in univariate analysis only.
Conclusion: MRSA transmission in the hospital remains low. However, MUO carriers are per definition not isolated until detection, which increases the risk of transmission. Also, transmission by not isolated carriers was higher on inpatient than outpatient wards and seemed (univariate) related to a higher intensity of care. Not isolated MRSA carriers were MUO as well as MKO, demonstrating that absent or delayed information at admission remains a key issue.

P120

Antimicrobial resistance and the use of selective decontamination of the digestive tract in intensive care units: an ecological study

A.K. van der Bij[1], A. Houben[1], E.A.N. Oostdijk[2], P.H.J. van der Voort[3], M.J.M. Bonten[2], J.C.M. Monen[1]
[1]*RIVM, CIb-EPI, Bilthoven,* [2]*University Medical Centre, Dept. of Medical Microbiology, Utrecht,* [3]*Onze Lieve Vrouwe Gasthuis, Dept. of Intensive Care Medicine, Amsterdam*

Introduction: Selective oropharyngeal decontamination (SOD) and selective decontamination of the digestive tract (SDD) are associated with improved patient outcome. However, possible disadvantages are their effects on the bacterial ecology in intensive care units (ICUs). In this study, we assessed rates of resistant isolates in ICUs with and without SOD or SDD, i.e., standard care (SC), and determined time trends in gram-negative bacteria using routine Dutch surveillance data.
Methods: All isolates of *Enterobacteriaceae* spp., *P. aeruginosa* and *Acinetobacter* spp. of blood specimens and clinical lower respiratory tract specimens of ICU patients were selected from the Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR), which collects antimicrobial susceptibility data of routinely cultured bacterial species from Dutch laboratories. To avoid multiple sampling we only selected the last isolate per species per patient per year (i.e., cumulative resistance) from 2008-2011. Information on SOD and SDD on ICU level were collected by a structured online questionnaire. Resistance rates per 100 bed to colistin, tobramycin, ciprofloxacin, ceftazidime and cefotaxime/ceftriaxone were calculated for all species combined and multilevel Poisson analyses, adjusted for ICU level and year, was used to determine differences in rates.
Results: The study included 44 ICUs, 600 blood isolates and 6983 respiratory isolates. Overall, from 2008-2011, there were no differences in the rate of isolates resistant to any of the antimicrobials between the ICUs, except for colistin (SOD versus SC, rate ratio (RR) 2.2; 95% confidence interval (CI) 1.4-3.5; SDD versus SC: RR 1.5; 95% CI 1.0-2.4). For all antimicrobial agents there was a decreasing time trend in the rate of resistant isolates in ICUs with SOD or SDD and in ICUs with SC, and when analyzing 2011 data only, there were no longer differences present in the rate of isolates resistant to colistin between ICUs with SOD or SDD and ICUs with SC.
Conclusion: Based on our data, we (1) showed no differences in rate of resistant isolates between ICUs with SOD, SDD or SC and (2) there was no increase in resistance over time in ICUs with SOD or SDD. Although our study is limited by its ecological nature and the lack of background data on ICUs other than ICU level, it adds to the evidence that the effects of SOD and SDD are limited in countries with a low level of resistance.

P121

The Ao value and thermal disinfection of bedpans: VRE and OXA-48 outbreak strains in relation to the European ISO 15883-3 guideline requirements

L.B.J. van der Velden[1], A. van Leeuwen[2], M. Wind-Bohne[2], A. Voss[2], M.H. Nabuurs-Franssen[2]
[1]*Radboud University Nijmegen Medical Centre, Dept. of Medical Microbiology, Nijmegen,* [2]*Canisius Wilhelmina Hospital, Nijmegen*

Introduction: Most hospitals use bedpan washer-disinfectors for the mechanical cleaning and thermal disinfection of reusable bedpans. The European guideline ISO 15883-3 states that washer-disinfectors have to achieve a minimum A_o value of 60 for the appropriate disinfection of reusable bedpans. This A_o value corresponds to a time equivalent in seconds at 80 °C. An A_o of 60 is viewed as an acceptable minimum for devices coming into contact with intact skin, such as bedpans, and corresponds with a disinfection temperature of 80 °C maintained for 60 seconds or of 90 °C maintained for 6 seconds. In contrast, for surgical instruments an A_o value of 600 is considered the minimum requirement.

During 2012, the Canisius Wilhelmina Hospital (CWH) in Nijmegen as well as several other hospitals in the Netherlands were faced with a vancomycin-resistant *Enterococcus faecium* (VRE) outbreak. Since previous data have shown that some *E. faecium* strains survive for 60 seconds at 80 °C while others do not, we determined the A_o value of the VRE outbreak-strain of the VRE outbreak in the CWH as well as the A_o value of the outbreak-strain of the OXA-48 *K.pneumoniae* outbreak in a Dutch hospital during 2011.

Materials and methods: Both the VRE and OXA-48 *K.pneumoniae* outbreak strain were tested in duplo to determine the minimum A_o value that results in the killing of all isolates. Overnight broth cultures (logarithmic phase cultures) and emulsified colonies (stationary phase cultures) were held at 65, 75 and 80 °C and samples for viable counts were obtained after 1, 2, 3 and 10 minutes at each temperature.

We determined the A_o value of all 38 bedpan washer-disinfectors used in the CWH. When the measured A_o value of a washer-disinfector was close to the minimal required A_o of 60, VRE PCR and cultures were performed on swabs of bedpans after disinfection by these washer disinfectors.

Results: The VRE outbreak strain survived 2 minutes at 80 °C (A_o = 120), and the OXA-48 *K.pneumoniae* survived 1 minute at 80 °C (A_o = 60). A minimum A_o value of 180 and 120 respectively, would be required for the adequate killing of these pathogens.

All 38 washer-disinfectors in use in the CWH achieved A_o values of > 60, and therefore functioned in agreement with the European guideline, although the lowest A_o value was only 73.

On bedpans disinfected by these washer-disinfectors, VRE was identified by PCR and cultures obtained by swabs.

Conclusion: Both the VRE and the *K.pneumoniae* OXA-48 outbreak strains we tested survived the 80 °C for 1 minute (A_o value = 60) that is required as a minimum for disinfection of bedpans according to the European guideline ISO 15883-3. Furthermore, VRE were identified by PCR as well as cultures of bedpans that had been cleaned by a washer-disinfector that functioned in agreement with this guideline. We suggest, at least during a VRE or other heat resistant pathogen outbreak, to increase the minimal acceptable A_o value of washer-disinfectors to at least 180.

P122

CXCL-13 in CSF of HIV-positive patients suspected of neurosyphilis

L.B.J. van der Velden[1], M.M. Verbeek[1], P.P. Koopmans[1], W. Ang[2], F.F. Stelma[1]

[1]*Radboud University Nijmegen Medical Centre, Dept. of Medical Microbiology, Nijmegen,* [2]*VU University Medical Centre, Dept. of Medical Microbiology & Infection Control, Amsterdam*

Introduction: The clinical presentation of neurosyphilis (NS) varies from asymptomatic to uveitis, otosyphilis, meningovascular syphilis, tabes dorsalis and general paresis.

Only two statements about diagnosing NS based on CSF properties are generally accepted: a positive CSF RPR is a sufficient criterium for the diagnosis of NS and a negative CSF treponemal test excludes NS. All other criteria lack sufficient evidence. To establish the NS diagnosis in HIV-positive patients, the 2008 IUSTI/WHO European guideline on the management of syphilis advices to either consider a positive CSF TPPA in combination with > 10 cells/mm^3 NS, or to use the TPHA index (of Vienna), although the authors also state that these criteria have generally not been validated in HIV-positive patients. The medical website uptodate provides a flowchart composed by Christina Marra, an authority on NS, which results in NS treatment for all HIV-positive patients with > 20 cells/mm^3 or 6-20 cells/mm^3 in combination with CD4 < 200/L or HIV RNA < 50 c/ml or when the patient is taking antiretroviral therapy. The Dutch STD guideline advices to only use the TPHA index in HIV-negative patients. This guideline suggests to determine HIV RNA in the CSF. Since HIV itself can result in CSF abnormalities, the absence of HIV makes NS more likely.

CSF CXCL-13 has been identified as a diagnostic tool in neuroborreliosis during recent years. Only 1 article about the possible value of CSF CXCL-13 in the diagnosis of NS has been published. We determined CSF CXCL-13 titers in the CSF of asymptomatic and symptomatic HIV-positive patients to investigate if this titer is helpful in the diagnostic workup of NS.

Methods: CXCL-13 was measured in the CSF of 19 HIV-seropositive patients, 5 symptomatic and 14 asymptomatic. The TPHA index (Vienna) was calculated for all patients: CSF TPPA/ Q albumin.

Results: All 3 RPR positive patients had high CXCL-13 titers. Of the 5 symptomatic patients, 1 was TPHA index positive, with a positive CSF RPR and high CXCL-13 titer. The other 4 were TPHA index negative and had low CXCL-13 titers. CSF leukocyte counts were: < 5 cells/mm^3 in 1 patient, 7 cells/mm^3 in the second patient and > 20 cells/mm^3 in 2 patients.

Of the 14 asymptomatic patients, 4 had a positive TPHA index: 2 were CSF RPR positive, both with high CXCL-13 titers; the other two had 0 and 16 cells/mm^3 as CSF leukocyte count respectively. All 10 TPHA index negative patients had low CXCL-13 titers, 8 with < 5 cells, 1 with 6 and 1 with 50 cells/mm^3.

Conclusion: CSF CXCL-13 may help discriminate between high CSF cell counts due to HIV itself from asymptomatic neurosyphilis, and may thereby help preventing unnecessary treatment for this difficult to diagnose disease.

P123

Clinical mumps cases with negative mumps diagnostics: timing of sampling or alternative agent?

A.L. Klink[1], J.E. van Steenbergen[2,3], J.J.C. de Vries[1]

[1]*Leiden University Medical Centre, Medical Microbiology, Leiden,* [2]*Centre of Infectious Diseases, LUMC, Leiden,* [3]*Centre for Infectious Disease Control, National institute for Public Health, Bilthoven*

Introduction: Despite universal vaccination against mumps since 1987, an ongoing mumps epidemic occurred in young adults in the Netherlands from 2009 to 2012.

During this epidemic, a striking proportion of patients with symptoms suggestive for mumps, the diagnostics turned out negative. The objective of this study was to explain the large amount of clinical mumps cases with negative mumps diagnostics.

Methods: Serological and PCR samples from clinical mumps cases sent to the LUMC for mumps diagnostics, from 2006 and august 2012, were retrieved by means of a GLIMS (laboratory information system) search. The search included patients from 4 neighbouring hospitals for which LUMC performed serology. PCRs, up to 2012, were performed at the RIVM and were retrieved. Additionally, serological and respiratory samples from clinical mumps cases sent to the Reinier the Graaf hospital (RDGG) in the same time period were retrieved.

The search covered patient characteristics, clinical data, sample information and other tests/ diagnosis.

Sensitivity and specificity of mumps serology were calculated with as gold standard mumps PCR with adequate timing (sputa, saliva, buccal or throat swabs taken within 7 days of first day of illness). For patients with adequately timed sampling (serology after 5 days of illness) and negative results on both tests, the following tests were performed: EBV and CMV serology and PCRs for influenza, parainfluenza and enterovirus.

Results: In total 1215 tests of 935 clinical mumps cases were sent to the LUMC (1084 tests) including the RDGG (131 test), of which 855 serological tests (135 IgG and 720 IgM & IgG) and 229 PCRs. The median age was 24 years, 22 in PCR and 25,5 in serology patients. 703 IgM results and 174 PCRs were negative.

Of patients with parotitis, mentioned in the clinical data field (n = 127), 49% of all PCR samples were drawn in the optimal time frame (< 7 days after onset of illness), 3% after one week, of 48% this was unknown. In total 16% of serology, of patients with parotitis (n = 144) was obtained within the optimal timeframe (> 5 days after onset), 31% outside and 53% unknown.

When including the inadequately timed samples in the analysis, the sensitivity of IgM testing in this study was 19% (2/11), and specificity 100% (12/12). If sampled adequately, sensitivity was 1/2 (50%) with a specificity of 3/3 (100%). Sensitivity and specificity of mumps IgM in vaccinated patients were 1/1 (100%), compared to 1/2 (50%) and 2/2 (100%) respectively in unvaccinated patients.

In total 17 clinical mumps cases with adequately timed negative PCR were additionally analysed, of whom 1 tested enterovirus and 1 parainfluenza 2 positive. In total 11 clinical mumps cases with adequate negative serology were additionally analysed, of whom none had CMV and/or EBV.

Conclusion: Mumps diagnostics seems to be negative in a significant portion of clinical mumps cases, not due to an alternative epidemic, but due to sampling outside of the appropriate timeframe for the specific test.

To improve the diagnostic tests of mumps, clinicians should be instructed which test in which patient and on which day.

P124

Human Herpesvirus-6 in cerebrospinal fluid: an innocent bystander or a serious pathogen?

A.L. Klink, A.C.T.M. Vossen, E.C.J. Claas

Leiden University Medical Centre, Dept. of Medical Microbiology, Leiden

Objectives: Human Herpesvirus-6 (HHV-6) has a seroprevalence of over 95%. It can cause exanthema subitum in young children and is associated with limbic encephalitis in immunocompromised patients. After infection, HHV-6 DNA integrates in cellular DNA, which might explain the presence HHV-6 DNA in clinical samples of patients with nonspecific causes of cell damage. The objective of this study was to determine whether HHV-6 DNA is found in CSF as an innocent bystander or a true pathogen.

Methods: CSF samples, from December 2011 till February 2012, were tested for HSV1-2, VZV and HHV-6 DNA, comparing patients with a clinical suspicion of a HHV-6 infection with patients with other infections or immunological diseases (controls).

Magna Pure LC, with a total nucleic acid kit, was used to extract DNA. Viral DNA, HSV (glycoprotein B gene, UL27), VZV (glycoprotein B gene, ORF 31) and HHV-6 (U31 gene), was detected with an in-house real-time PCR.

From patient files, if accessible, symptoms, diagnosis, CSF cell count and MRI findings were obtained.

Results: Of 153 patients, 165 samples of CSF were tested. In total, seven HSV-1, two HSV-2 and six VZV positive samples were detected. In six patients (seven samples) the HHV-6 PCR was positive, all were clinically suspected of HHV-6, all controls were negative.

HSV-1 was detected in four patients with previously diagnosed HSV-1 encephalitis, in two patients with a clinical suspicion of acute infectious polyneuropathy and in a patient with trigeminal nerve neuropathy.

The samples with HSV-2 were also detected in routine PCR diagnostics, of which one patient presented with relapsing viral meningitis and the other with limbic encephalitis.
Of the six patients with VZV DNA five had a clinical picture of VZV meningitis. One patient had a dubious positive signal, Cq 41.8, and was diagnosed with Multiple Sclerosis.
The HHV-6 positive patients were a demographically and clinically heterogeneous group. Among them was a neonate who died of a systemic candida infection and two children aged six and seven, one of whom died, without further clinical information. The fourth patient received chemotherapy for breast cancer and presented with a status epilepticus and MRI findings consistent with limbic encephalitis. An adult male who had received stem-cell transplantation was diagnosed with limbic encephalitis, possibly of auto-immune origin, also tested positive. The sixth patient, a male aged 82, presented at the emergency department with self-limiting headache and confusion.
Conclusion: In this study HHV-6 could only be found in a small number of CSF samples and could not be correlated to other infections. HHV-6 DNA was only detected in either very young patients without or adult patients with a clinical picture of limbic encephalitis. Because HHV-6 could not be detected in MS patients HHV-6 might not be an innocent bystander. On the other hand, the finding of HHV-6 in a patient with short-term transient complaints and the differential diagnosis of autoimmune inflammation in the other patients suggests that HHV-6 still might be just an innocent bystander. Therefore, the significance of HHV-6 in CSF remains unclear.

P125

Outbreak of a t2330 livestock-associated methicillin-resistant *Staphylococcus aureus* in acute care hospitals in the northern Netherlands

M. Köksal[1], J.W.A. Rossen[1], J.P. Arends[1], M. Lokate[1], J.A.C. van der Weerd[1], A.J.L. Weersink[2], S.O. Axson[2], A.J. Sabat[1], G.A. Kampinga[1], A.W. Friedrich[1]
[1]*Universitair Medisch Centrum Groningen, Medische Microbiologie, Groningen,* [2]*Meander Medisch Centrum, Medische Microbiologie, Amersfoort*

Introduction: A patient from a regional hospital, temporarily admitted to a University Medical Center (UMCG) for specialized treatment of bullous pemphigoid, appeared positive for methicillin-resistant *Staphylococcus aureus* (MRSA). Therefore, MRSA screening of patients and staff was performed in the two hospitals and isolates were characterized in more detail.
Methods: From patients and healthcare workers (HCWs) in the two hospitals who had been in contact with the index patient, nose and throat and from patients also perineum and wound swabs were taken for screening of MRSA.
Each MRSA positive sample was characterized in more detail by determining its resistance pattern using vitek 2 and by molecular typing using a DNA micro-array (clondiag) and spa-typing.
Results: Spa-typing identified the MRSA from the index patient as spa-type t2330. The based upon repeat pattern (BURP) analysis revealed that this spa type belongs to livestock-associated MRSA. This spa-type was found before in Belgium, Germany and the Netherlands, counting worldwide for 0.01% of all submissions on the Ridom SpaServer.
The MRSA screening in the UMCG revealed one HCW that had performed wound care of the index patient to be positive for MRSA, spa-type t2330. Subsequently, patients that had been in contact with this HCW were screened again, also taking wound swabs if applicable, this time. One patient that was negative during the first screening (during which only nose, throat and perineum swabs were taken) appeared to have an MRSA t2330 positive wound swab.
The MRSA screening among 20 patients and more than 50 HCWs in the regional hospital revealed three HCWs (one resident physician and two nurses, who have had contact with the index patient after his relocation to the regional hospital) to be positive for MRSA t2330.
All MRSA t2330 isolates found in the two hospitals had the same resistance pattern. DNA micro-array results of the isolates confirmed that they belong to the ST398. From an historical database containing data of MRSA found in the UMCG in the past ten years it appeared that spa-type t2330 was never found before in the UMCG.
Apart from the index patient, who had several 'relatives living on a farm', none of the carriers turned out to have specific risk factors in relation to livestock. Eradication therapy was not indicated in both patients, due to the presence of wounds. Eradication therapy was successful in all HCWs.
Conclusion: Our data show a nosocomial outbreak with a rare but apparently very tenacious and relatively easily transmittable MRSA, spa-type t2330, which belongs to the spa-clonal complex of livestock-associated MRSA. This shows that some livestock-associated MRSA seem to be transmittable from human tot human, even to HCWs. Reasons for this need to be understood in future studies. Screening of patients – including wounds – and spa-typing of all outbreak *Staphylococcus aureus* strains is strongly recommended for the early detection of such clones.

P126

Evaluation of Roche Cobas 6000 e601 electrochemiluminescence immunoassays for hepatitis B and C and human immunodeficiency virus compared to the AxSYM microparticle enzyme immunoassays

I.H.M. van Loo, M. Garcia Perdomo, N. Nadurinze, D. Ploum, V.J. Goossens
MUMC, Dept. of Medical microbiology, Maastricht

Introduction: In this study we compared the Roche Cobas 6000 e601 module electrochemiluminescence immunoassays for screening of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus 1,2 (HIV) with the AxSYM microparticle enzyme immunoassays for validation in routine use.
Methods: In total we selected 583 sera from our database of routine serum samples from patients. Selection of the samples was based on results of the AxSYM analyzer (Abbott). For each assay 50% of the selected samples were negative and 50% of the samples were positive. Among the positive samples we included approximately 10% samples that were considered to be false positives. We evaluated the following assays: HBsAg, HBeAg, anti-IgM HBcore, anti-HBcore, anti-HBe, anti-HBs, anti-HCV and anti-HIV/p24.
Results: In the Cobas HBsAg assay we observed agreement in 86% of the samples. Twelve out of 88 samples were positive in the AxSYM HBsAg assay and negative in the Cobas HBsAg assay. We considered these samples to be false positive in the AxSYM assay since the other serological HBV markers were negative. In total a reduction of more than 86% of false positive HBsAg tests was achieved by using the Cobas HBsAg assay. For the other HBV serological markers we observed agreement between both assays in 95% for HBeAg, 98% for anti-HBcore, 84% for IgM anti-HB core, 86% for anti-HBe and 83% for anti-HBs.
For HCV we observed agreement between both tests in 86% of the 90 samples. In 13 samples the AxSYM anti-HCV assay was borderline or positive in samples, which were negative in the Cobas anti-HCV assay. Two samples that were not confirmed by Western blotting remained positive in the Cobas anti-HCV assay. For HCV screening we observed a reduction of 86% of false positive results by using the Cobas anti-HCV assay.
For HIV screening overall agreement was 90%. Nine out of 99 samples were positive in the AxSYM anti-HIV/p24 assay and negative in the Cobas combi HIV PT assay. We considered these samples to be false positive since the VIDAS HIV screening (Biomrieux) was also negative. Among this sample selection all false positive AxSYM samples were negative in the Cobas combi HIV PT assay.
Conclusion: Screening of HBsag, HCV IgG and HIV combi PT gives more than 80% reduction in false positive results using the Roche Cobas 6000 e601 assays.

P127

PCR for direct detection of the mosaic *Neisseria gonorrhoeae* penA gene in urines and cervical, rectal and tonsillar swabs

A.P. van Dam[1], L. Thiel[2], M. Dierdorp[2], S.M. Bruisten[2]
[1]*OLVG, Dept. of Medical Microbiology, Amsterdam,* [2]*Amsterdam Health Service, Amsterdam*

Introduction: Emergence of diminished susceptibility to third-generation cephalosporins is worrying since these antibiotics are the only options left to treat gonorrhoea. Nowadays the gonorrhoea diagnosis is often obtained by NAAT, without culture, which precludes determination of antimicrobial resistance. The presence of a mosaic penA gene, partly derived from commensal *Neisseria* strains, is strongly associated with diminished susceptibility of *Neisseria gonorrhoeae* (Ng) against cephalosporins. We developed a direct PCR test to discriminate between the presence of this mosaic *penA* gene and the wild type gene.
Methods: Swabs and urines were obtained and stored in medium for NAAT testing (Tigris Aptima, GenProbe) from patients with gonorrhoea. Also Ng strains were obtained by culture on selective GC agar plates and subsequently stored at -80 °C. Presence or absence of a mosaic penA gene in these strains was demonstrated by a PCR as described by Whiley et al.[1]
Results: Using one conserved forward primer and two reverse primers, specific for mosaic- and wild type penA genes, and SYBR green as a fluorescing agent, two new real-time PCRs were developed. Melting curve analysis showed that the T_m of the mosaic penA PCR product was 85, whereas the T_m of the wild type gene was 87 °C. Testing diluted DNA samples showed that the mosaic penA gene PCR was 10-100 fold more sensitive than the wild type gene PCR. Both PCRs were negative with strains belonging to *N. meningitidis* (n = 3), *N. lactamica* (n = 4), *N. subflava* (n = 2), *N. cinerea* (n = 1) and *N. elongata* (n = 1). Ten urine (U), 10 cervical (C), 10 rectal (R) and 10 tonsillar (T) samples, all negative in the NAAT for Ng, were negative in both PCRs. Paired samples from patients (n = 36), who had a positive culture and NAAT (10 R, 9 U, 8 C, 9 T), were tested in the mosaic- and wild type penA PCR. The result was concordant in 35/36 samples: 4 pairs tested positive in the mosaic PCR and 31 in the wild type PCR. From one patient a wild type strain had been cultured from the throat, but both penA PCRs on the swab were negative, possibly due to a low amount of DNA in the sample. To assess the prevalence of the mosaic penA gene in patients with *pharyngeal* gonorrhoea, 70 consecutive Ng-NAAT positive tonsillar swabs were tested. Four samples were positive in the mosaic penA PCR, and 50 in the wild type penA PCR, suggesting a prevalence of the mosaic penA gene of 7.4% in this STI population.
Conclusion: We successfully developed discriminating PCRs with which the Ng mosaic penA gene can be detected without culture of Ng. This test can be used to estimate the prevalence of diminished susceptibility of Ng against cephalosporins in regions where culture is no longer performed.

References

- Whiley, et al. Pathology. 2007;39:445-6.

P128

Characterization of the humoral response of Wayampi Indians from French Guyana directed against *Staphylococcus aureus*

B.C.G.C. Slingerland[1], R.R. Ruimy[2], M.C.V. Vos[1], W.J.B. van Wamel[1]

[1]*Erasmus MC, Dept. of Medical Microbiology, Rotterdam,* [2]*CHU Bichat-Claude Bernard, Service de Bacteriologie, Paris, France*

Introduction: *Staphylococcus aureus* (*S. aureus*) nasal carriage in humans increases the risk of getting endogenous infections. Approximately 30% of the population is a persistent carrier of *S. aureus*, 40% an intermittent and 30% are non-carriers. Interestingly, in the Netherlands we observed minimal differences in the humoral response directed against *S. aureus* between the different carriage patterns. At this moment it is still unclear if people from different parts of the world share the same antibody responses to *S. aureus*. In addition, if these responses are different, could this be due to exposition to *S. aureus* with other genetic backgrounds? Therefore, we studied serum samples from an isolated group of Wayampi Indians, living in French Guyana, healthy Sudanese volunteers living in Khartoum and healthy Dutch volunteers for IgG and IgA antibody responses directed against *S. aureus*.

Methods: Serum samples from 151 Wayampi Indians, 55 Sudanese healthy human volunteers and 20 Dutch healthy human volunteers were analyzed for IgG and IgA antibodies directed against 40 *S. aureus* antigens using a multiplex assay based on a bead-based flow cytometry technique. Experiments were performed in independent duplicates, and the median fluorescence intensity (MFI) values, reflecting semiquantitative antibody levels, were averaged. In each experiment, control beads (no protein coupled) were included to determine nonspecific binding. Anti-staphylococcal antibody levels in the different groups were compared.

Results: The individual antibody responses in the Wayampi Indian population varied extensively, this was also observed in the Dutch and Sudanese healthy volunteer cohort. For most of the antigens, significant differences were found in both IgG and IgA antibody levels between the Wayampi Indians on the one hand and the Dutch and Sudanese healthy volunteer cohorts on the other hand. In specific IgG and IgA antibody levels directed against *S. aureus* antigens alpha toxin, CHIPS, ETA, ETB, SCIN, TSST-1, SEA, SEB, SEH and SEQ differed significantly from the Dutch and/or Sudanese healthy volunteer cohort. Interestingly, these antigens are all located on mobile genetic elements.

Conclusion: Wayampi Indians show a different humoral response directed against *S. aureus* compared to Dutch and Sudanese healthy human volunteers.

P129

The CROCUS-study – Consequences and risk factors of congenital cytomegalovirus infection

M.J. Korndewal[1], A.M. Oudesluys-Murphy[1], H.J. Boot †[2], A.C.M. Kroes[1], M.A.B. van der Sande[2], A.C.T.M. Vossen[1], H.E. de Melker[2]

[1]*Leiden University Medical Center, Dept. of Medical Microbiology, Leiden,* [2]*National Institute for Public Health and the Environment, Laboratory of Infectious Diseases, Bilthoven*

Introduction: Congenital cytomegalovirus (CMV) infection is the most common congenital infection worldwide, with a birth prevalence of around 0.5% in the Netherlands. At birth symptoms such as splenomegaly, microcephaly and intracranial calcifications, occur in approximately 10 to 15% of the cases.

Almost half of these symptomatic children and 10 to 15% of the initially asymptomatic children will develop long term sequelae (predominantly sensorineural hearing loss) during the first years of life.

Data on long term sequelae beyond the age of 5 years are scarce. With this study we aim to obtain data on the disease burden of congenital CMV infection, including long term sequelae of congenital CMV infection, in Dutch children aged 5 to 6 years.

Methods: 75.000 parents will be asked to give informed consent to test the dried blood spot of their child for congenital CMV infection, using PCR. We expect to test 25.000 dried blood spots of 4 to 5 year old children.

In this way we expect to detect approximately 125 children with a congenital CMV infection. These children and a control group (twice as large) will be asked to participate in the second part of the study.

In the second part data on hearing, cognitive development, vision and motor development of these children will be retrospectively collected from medical records, records of the Youth Health Care Organizations, parent questionnaires and schools.

Results: Of the 28 Municipal Health Services in the Netherlands (comprising the Youth Health Care Organizations) 19 were willing to participate in this study. Four organizations were not able or not willing to participate.

Up to this moment, 43.6% of the 44791 parents who have been asked to give informed consent to test their child's dried blood spot, have consented. Around 1% of the parents objected to testing. The total response rate was 44.7 %.

Conclusion: 1. The response rate is expected to be sufficiently high to include about 125 children with congenital CMV in this study. This should be sufficient to enable the detection of significant difference in the prevalence of sensorineural hearing loss between those with and without a congenital CMV infection.

2. This study will generate knowledge on the disease burden of congenital CMV in the Netherlands at the age of 5 or 6 years. This can be used estimate the potential impact of primary prevention by vaccination or secondary prevention by screening.

P130

Evaluating MALDI-TOF MS as method to determine relatedness of isolates in comparison with traditional MLST

J.C. Sinnige, C.H.E. Boel, J.G. Kusters

UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Objectives: We evaluate the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as a method to quickly determine the relatedness of isolates contributing to an outbreak. Traditional multi-locus sequence typing (MLST) is compared with protein spectrum based typing.

Methods: A total of 245 vancomycin resistant *Enterococcus* (VRE) outbreak isolates were retrieved from our collection. Additionally 90 isolates from different origin (individual clinical cases, community surveillance studies, animal and environmental origin) were included and spectra were obtained using a Bruker MALDI Biotyper system. All strains were typed using multi-locus sequence typing (MLST) as the classical reference method and compared with MALDI-TOF MS based typing. The different typing methods were evaluated using BioNumerics version 7.0 software created by Applied Maths NV. Peaks common to all *Enterococcus faecium* species were excluded and only discriminating peaks were used for clustering.

Results: MALDI-TOF spectra were compared by performing pearson proportion analysis on the main spectral projections (MSPs) and relations were visualized in a dendogram. Although not fully concordant with MSLT typing, MALDI-TOF MS analysis did reveal that the majority of outbreak strains clustered together. In addition a clear subclustering of strains from individual hospitals was observed. An ANOVA test was used to determine the discriminating power of individual mass peaks. This revealed a subspectrum of 46 peaks that provides an optimal matching for concordance with MSLT typing (p-value < 0.05).

Conclusion: MALDI-TOF MS can provide rapid information when investigating the relatedness of different isolates. However, there were some differences in clustering between the two typing methods hence additional testing is required to get more insight in the potential value of MALDI-TOF MS based typing.

P131

IL-36 cytokines: new IL-1 family members that balance T-cell responses against *Aspergillus*

M.S. Gresnigt, B. Roesler, C.W.M. Jacobs, K.L. Becker, L.A.B. Joosten, J.W.M. van der Meer, M.G. Netea, C.A. Dinarello, F.L. van de Veerdonk

Radboud University Nijmegen Medical Centre, Experimental Internal Medicine, Nijmegen

Interleukin-1 family cytokines are key players in the induction of T-helper cell responses. Recently, new IL-1 family members, the IL-36 cytokines, were discovered. These IL-36 cytokines exhibit properties that are similar to those of IL-1 and play a prominent role differentiating nave T-cells to specific lineage T-cells. Furthermore the IL-36 cytokines were found to a role in neutrophil influx to the airways. Since, T-helper responses and their role in neutrophil recruitment play a key role in the host defense against invasive pulmonary aspergillosis we investigated the role of the novel IL-36 cytokines in *Aspergillus fumigatus*-induced human T-helper cell responses.

By stimulating PBMCs from healthy volunteers with different morphological forms of *A. fumigatus* we observed that this fungus can variably increase steady-state mRNA levels of all IL-36 subfamily members. While IL-36a was not strongly induced by any morphological form of *Aspergillus*, IL-36 and IL-36 receptor antagonist (IL-36Ra) were induced by heat inactivated (HI) *A. fumigatus* resting conidia. Most strikingly live germinating conidia or heat inactivated hyphae proved to be strong inducers of IL-36? with 17-fold and 76-fold increase in mean gene expression respectively, while these forms also induced IL-36 and IL-36Ra.

We found that supplementation of PBMCs stimulated with *A. fumigatus* with recombinant human IL-36 receptor antagonist (IL-36Ra) drastically altered T-cell responses. The exogenous IL-36Ra was able to suppress *Aspergillus*-induced Th17 and Th1 responses, but did not modulate innate, Th2 and regulatory T-cell responses. However, the biological relevance of IL-36 signaling pathway is demonstrated by the fact that blockade of endogenous IL-36Ra boosted *Aspergillus* stimulated IL-17 and IFN-γ production by PBMCs.

Collectively we demonstrated that *Aspergillus* can activate the IL-36 receptor pathway which is in turn actively involved in regulating the *Aspergillus*-induced Th1 and Th17 responses. As this provides evidence that this novel cytokine pathway can play an important role in host defence, future research is warranted to extend the knowledge on the role of IL-36 in the host defense against *Aspergillus fumigatus*, and other human pathogens.

P132

Discriminating complicated from uncomplicated *Staphylococcus aureus* bacteraemia: a genetic and phenotypic study

J.W. Swierstra

Erasmus MC, Dept. of Medical Microbiology & Infectious Diseases, Rotterdam

Background: *Staphylococcus aureus* (*S. aureus*) causes many types of infection, of which bacteraemia is amongst one of the most lethal. Bacteraemia occurs in 2 types: relatively harmless uncomplicated bacteraemia and complicated bacteraemia, which often leads to longer hospitalization, increased antibiotics use and secondary infections. In this study we would like to determine if there are risk factors which determine whether a bacteraemia will become complicated.
Methods: 126 *S. aureus* bacteraemia patients in the Erasmus MC were identified retrospectively from August 2009 to August 2011, and infecting strains and epidemiological data were collected. The PFGE type for all strains was determined. Also, the MIC against flucloxacillin, vancomycin and augmentin and biofilm formation with and without antibiotic pressure was determined for a restricted cohort of 15 strains isolated form well-documented complicated bacteraemia and uncomplicated bacteraemia.
Results: Patient characteristics do not show new risk factors for obtaining complicated bacteraemia. *S. aureus* strains isolated from complicated and uncomplicated *S. aureus* bacteraemia are evenly distributed among the different PFGE types and do not cluster. MIC values also do not vary between complicated and uncomplicated strains, as all strains are susceptible. Biofilm production without antibiotic pressure is similar between both groups, although differences in growth medium are observed. Interestingly *S. aureus* isolates from complicated bacteraemia show more biofilm production when grown in 1 x MIC vancomycin compared to uncomplicated strains ($p < 0.05$).
Conclusion: Discriminating *S. aureus* isolated from complicated and uncomplicated bacteraemia remains difficult, although biofilm formation during antibiotic challenge does seem indicative of complication.

P133

Prospective screening for the prevalence of the novel mecA homologue (mecC, the Skov variant) in MRSA using real-time PCR

R.H.T. Nijhuis, N.M. van Maarseveen, A.A. van Zwet

Rijnstate, Medical Microbiology and Immunology, Velp

Objectives: Since the introduction of MRSA, rapid and accurate detection of individuals infected or colonized with MRSA is of high importance to prevent the spread of these bacteria. In 2011, anovel mecA homologue (nowadays mecC) primarily found in cattle was described by Garcia-Alvarez et al. MRSA strains harbouring this novel homologue were phenotypically resistant to methicillin, but tested negative in assays based on the mecA gene since there is only 70% homology with the known mecA gene. Although retrospective studies showed a very low prevalence of this novel mecA variant (11 in 12691 clinical isolates, Cuny et al; 2011), we altered our MRSA detection approach by including a real-time PCR targeting mecC. This is because of the many farms with livestock of cows and pigs in our region. In this study, we describe the results of an extensive prospective screening for MRSA containing the mecC gene during a period of 11 months.
Methods: All samples received for MRSA detection were incubated overnight in a selective broth, after which two internally controlled real-time PCRs were performed. One of these real-time PCRs is targeting the resistance genes mecA and mecC, while the second real-time PCR detects a *Staphylococcus aureus* specific gene (Martineau et al). Samples with a Ct-value < 39 for the Martineau-gene together with any signal for either mecA and mecC are considered MRSA suspected. Confirmation of MRSA suspected broths is done by either culture, using the Brilliance MRSA agar (Oxoid) (samples from patients visiting a general practioner) or by a second real-time PCR, the GeneOhm MRSA (BD) (patients not from a general practioner). In the latter case, positive GeneOhm MRSA results were further confirmed by culture. Moreover, any broth with a signal for mecC is confirmed using both culture and the GeneOhm MRSA assay. Samples positive for MRSA were further characterized with spa-sequence typing.
Results: In a period of 11 months, 3509 broths were tested for the presence of MRSA containing either the mecC or the mecA gene. In none of the broths tested mecC was detected, whereas MRSA containing mecA was found in 1.85% (65) of all samples.Twenty-seven of these samples came from patients visiting a general practioner, from 21 different patients. The remaining 38 MRSA isolates originated from 27 unique patients (non-general practioners). Spa-type 011 (34.7%) was the most common MRSA in both groups, followed by spa-type 899 (10.2%) and 17 other miscellaneous types.
Conclusion: In this study, we describe a prospective screening for MRSA containing the mecC gene. Despite the high prevalence of cattle-related MRSA in our region, no mecC-containing MRSA was detected. Although the number of MRSA positive samples was relatively low, the prevalence of mecC MRSA seems to be rare.

P134

Detection of *Coxiella burnetii* in goat semen by PCR

M. Heijne[1], M.V. Kroese[1], B.J. van Rotterdam[2], M. Koene[1], H.I.J. Roest[1]

[1]CVI, part of Wageningen UR, Bacteriology and TSE 's, Lelystad, [2]RIVM, Bilthoven

Introduction: *Coxiella burnetii* is the etiologic agent of Q fever. In the 2007-2010 Dutch Q fever outbreak dairy goats were the cause of human infection. Little is known about the transmission routes within goat herds. Although aerosol transmission is thought to be the major infection route, no information is available on the risk of sexual transmission. This might be an important route as one male goat mates about thirty to sixty female goats. To investigate the possibility of sexual transmission we investigated semen of male goats on Q fever positive farms.
Methods: Semen samples of nine goats from three different farms were collected in the summer and autumn of 2010. The farms were bulk tank milk positive for *C. burnetii* DNA and all male goats were vaccinated against Q fever during spring 2010. Eight goats were tested three times with an interval around six weeks. One goat was only tested once. Samples were part of a voluntary monitoring program for Q fever in semen due to governmental regulations. A *C. burnetii* specific multiplex real-time PCR was used to test the samples. This PCR was validated for use in semen and targets IS1111. The IS1111 element is a multicopy gene, making this PCR very sensitive.
Results: Semen samples have been tested from June 2010 till December 2010. PCR results were all negative.
Conclusion: Our results suggest that the risk of shedding of *C. burnetii* in semen of vaccinated goats is small. No information was available about the infection status of these goats. In female goats and cattle the infection status prior to vaccination influences excretion of *C. burnetii*. Vaccination of infected animals does not prevent shedding of *C. burnetii*. Since nothing is known about the risk of excretion of *C. burnetii* in semen of goats, we believe it is important to share our data. Additional research with vaccinated and non-vaccinated male goats is needed.

P135

Adaptive immune responses determining influenza outcome in patients with hematologic malignancies

J. Gooskens[1], W.A.F. Marijt[1], E.H.R. van Essen[1], G.F. Rimmelzwaan[2], A.C.M. Kroes[1]

[1]Leiden University Medical Center, Dept. of Medical Microbiology, Leiden, [2]Erasmus Medical Center, Dept. of Virology, Rotterdam

Introduction: Patients with hematologic malignancies develop a wide clinical spectrum during prolonged influenza virus infection. We assessed the role of adaptive immune responses in determining outcome differences.
Methods: Consecutive case series describing four patients with hematologic malignancies admitted to the hospital with =14 days prolonged 2009 influenza A (H1N1) virus infection. Laboratory tests included molecular detection of influenza virus and neuraminidase gene H275Y mutation conferring oseltamivirresistance, and assessment of lymphocyte subsets, virus-specific antibody titers and T-cells. We assessed clinical and virological outcome measures during prolonged viral excretion and evaluated humoral and cellmediated immune findings as determinants.
Results: All four patients displayed prolonged viral excretion (range, 29-49 days) during T-cell lymphopenia. Three patients developed severe viral lower respiratory tract infection in the absence of virusspecific antibodies or NK-cells. One patient experienced mild symptoms during simultaneous presence of antibodies and CD16+ cytotoxic NK-cells which suggested that antibody-dependent cell-mediated cytotoxicity responses provided clinical protection. Virus-associated mortality manifested during sustained profound lymphopenia with inability to mount antibody responses (2 patients) whereas viral clearance correlated with proliferative virus-specific T-cell responses (2 patients).
Conclusion: Humoral and cell-mediated adaptive immune responses appear major outcome determinants during prolonged 2009 H1N1 virus infection among patients with hematologic malignancies.

P136

HIV-1 co-receptor prediction values differ significantly between plasma and cerebrospinal fluid

A.J. Stam[1], M. Nijhuis[2], P.M. Ham[2], T. Mudrikova[2], A.M.J. Wensing[2]

[1]Utrecht University, Dept. of Medical Microbiology, Utrecht, [2]University Medical Center Utrecht, Virology, Dept. of Medical Microbiology, Utrecht

Introduction: The CNS can be considered a distinct anatomical and physiological compartment, protected by the blood-brain barrier (BBB) and surrounded by cerebrospinal fluid (CSF). Within the CNS mostly perivascular macrophages and microglia are infected, in contrast in blood T-lymphocytes are main source of infection. As infection of macrophages and microglia occurs mostly via the CCR5 coreceptor, it has been suggested that neurotropism is associated with CCR5 coreceptor usage. Our aim is to asses to what extent these cellular and anatomical differences are associated with HIV-variation, in particular in the variable loop 3 (V3) of gp120.
Methods: Paired CSF and plasma samples were cross-sectional analysed in HIV-1 infected subjects with or without neurological symptoms. HIV-RNA levels, genotypic divergence in gag, pol and Gp120-V3 was assessed and co-receptor tropism was predicted using the $geno2pheno_{[co\text{-}receptor]}$ algorithm (FPR 10%). Spearman's rank correlation and the Wilcoxon signed rank test were

used to analyse viral characteristics and FPR values. Maximum likelihood phylogenetic trees were constructed (MEGA 5) to investigate evolutionary relationships.
Results: 42 paired samples from 21 HIV-1 infected patients were compared of whom 14 had neurological symptoms. All subjects were antiretroviral treatment nave (n = 14), interrupted therapy (n = 5), or very recently initiated treatment (< 2 months, n = 2). HIV-RNA levels were significantly higher in plasma than in CSF (Median HIV-RNA plasma 4.80 log c/ml and median HIV-RNA CSF 4.38, p = 0.028), yet showing positive correlation (ρ = 0.60, p = 0.004). Eight subjects (38%) had different HIV gag-pol amino acid sequences in CSF and plasma. Mutations were more often observed in CSF (6 samples) than in plasma (3 samples). Twelve subjects had R5-predicted virus in both compartments. One subject had X4 predicted virus in both compartments. Two subject had R5 predicted virus in CSF and X4 predicted virus in plasma. For the other subjects no co-receptor prediction could be determined in both compartments. FPR values were significantly higher in CSF than in plasma (p = 0.003).
Conclusion: In a population with limited drug selective pressure, subjects often show variation of HIV-1 quasispecies in CSF and plasma. Although nearly all patients harbored R5 predicted viruses in both compartments, we observed significantly higher values for predicted tropism (FPR) in CSF when compared to plasma. These data possibly reflect differences in target cell affinity of viruses replicating in the CNS as compared to plasma.

P137
The role of hydrogen peroxide resistance in pneumococcal lifestyle

C.A. Hinojosa[2], V. Farshchi Andisi[1], C.J. Orihuela[2], J.J.E. Bijlsma[1,3]
[1]*University Medical Center Groningen, Laboratory of Molecular Bacteriology, Dept. of Medical Microbiology, Groningen,* [2]*University of Texas Health Science Center at San Antonio, Dept. of Microbiology and Immunology, San Antonio, USA,* [3]*Intervet International BV, MSD Animal Health Discovery & Technology – Expression, Boxmeer*

Streptococcus pneumoniae is a gram-positive, catalase-negative bacterium and is classified as a facultative anaerobe. It colonizes the human nasopharynx, but can also cause serious disease. The bacterium is exposed to significant oxidative stress both from endogenously produced high levels of hydrogen peroxide (H_2O_2) during aerobic growth and from the host through the oxidative burst. How *S. pneumoniae* protects itself against oxidative stress is still unclear. Previously, we identified an operon, that plays a significant role in the survival of H_2O_2 stress *in vitro*. Furthermore, our analysis indicated that in a pneumonia mouse model it plays a role during the later stages of disease development. Subsequent analyses indicate that the operon also play a role in the survival of reactive nitrogen species To further dissect the role of hydrogen peroxide survival in the pneumococcal lifecycle we investigated the role of this operon in other important stages of the pneumococcal lifestyle such as desiccation stress, which may be important for transmission and biofilm formation, an important aspect of colonization. Deletion of the operon had a significant impact on biofilm formation and desiccation survival. In case of desiccation survival, we also identified another oxidative stress component that plays a significant role. Combined these results indicate that survival of H_2O_2 is important for *S. pneumoniae* at all stages of its lifestyle.

P138
Novel quantitative assays identify potent effects of artemisinins on gametocyte viability and transmission of human malaria

K. Dechering[1], M. Timmerman[1], G.J. van Gemert[2], M. van de Vegte-Bolmer[2], R.W. Sauerwein[1,2]
[1]*TropIQ Health Sciences B.V., Nijmegen,* [2]*Radboud University Nijmegen Medical Centre, Dept. of Medical Microbiology, Nijmegen*

Background: Eradication of malaria is critically dependent on the generation of novel intervention methods that block transmission of the parasite. Transmission depends on the generation of gametocytes, sexually differentiated forms of the parasite that initiate sporogonic development in the mosquito vector.
Methods: We set out to develop novel assays to monitor drug effects on the viability of gametocytes. Furthermore, we addressed whether a reduced viability impacted mosquito infectivity by using a modified protocol for the standard membrane feeding assay.
Results: Out of a set of known antimalarials, artemisinin-derived compounds showed potent effects on gametocyte viability. Interestingly, these compounds reduced viability but did not affect gametocyte number in an *in vitro* culture, as gametocytes could still be detected by microscopy. However, the reduction in gametocyte viability resulted in a reduction of subsequent development of oocysts in the mosquito. This reduction could in part be explained by an inability of drug-exposed parasites to form female gametes. Detailed dose response experiments showed that the sexual stages of the parasite are 20-50 fold less sensitive to artemisinins than the asexual stages.
Conclusion: We have developed a set of robust and quantitative assays to monitor drug effects on malaria transmission stages. These assays revealed potent effects on gametocyte viability of clinically relevant artemisinin-like drugs.

P139
Heterologous protection against malaria in a subset of volunteers after immunizations with *Plasmodium falciparum* sporozoites under chloroquine prophylaxis

R.W. Sauerwein[2], R. Schats[1], E.M. Bijker[2], A. Scholzen[2], A.C. Teirlinck[2], G.J. van Gemert[2], M. van de Vegte-Bolmer[2], L. van Lieshout[1], C.C. Hermsen[1], L.G. Visser[2]
[1]*Leiden University Medical Centre, Dept. of Infectious Diseases, Leiden,* [2] *Radboud University Nijmegen Medical Centre, Dept. of Medical Microbiology, Nijmegen*

Abstract not yet approved for publication.

P140
Immunization of healthy volunteers under chloroquine prophylaxis with different numbers of *P. falciparum* infected mosquito bites

E.M. Bijker[1], R. Schats[2], A.C. Teirlinck[1], A. Scholzen[1], C.C. Hermsen[1], L.G. Visser[2], R.W. Sauerwein[1]
[1]*Radboud University Nijmegen Medical Centre, Dept. of Medical Microbiology, Nijmegen,* [2]*Leiden University Medical Center, Dept. of Infectious Diseases, Leiden*

Abstract not yet approved for publication.

P141
Homogeneity in anaerobic yeast cultures at near-zero growth rate

M.M.M. Bisschops, M.A.H. Luttik, B. Rieger, J.T. Pronk, P. Daran-Lapujade
Delft University of Technology, Biotechnology, Delft

Non-growing but metabolically active yeast cultures are of great interest for industrial applications in which biomass is an undesired byproduct. Uncoupling growth and production would result in higher product yields. However, the usability of non-growing cultures depends on their robustness, e.g. their ability to resist to environmental stresses and maintain metabolic activity over prolonged periods of time. During prolonged cultivations, non-dividing cells in these cultures age and thereby also provide an attractive and powerful alternative model for the study of aging in higher eukaryotes. To obtain stable cultures of non-growing but metabolically active yeasts, the industrial and model yeast *Saccharomyces cerevisiae* was grown in retentostat cultivation.
In retentostat, *S. cerevisiae* cells accumulate in the culture vessel, but are continuously fed with a fixed medium supply. At high cell densities, the limited supply of glucose, sole energy and carbon source, leads to extreme calorie restriction, in which all available energy is invested in maintenance processes at the expense of cell division (doubling time is approximately 2 months). In retentostat, the non-dividing yeast cells become highly robust and retain high viability and metabolic activity for long periods of time (> 21 days).[1] Physiological and transcriptional analysis revealed many features of quiescent (i.e. carbon starved) cultures.[2,3] So far only whole population measurements had been performed in retentostat, and it is yet unknown whether the culture consists of a homogeneous, robust, yeast population, or is rather composed of a heterogeneous population in which the proportion of robust cells increases during the course of the retentostat.
Because robustness is related to quiescence, in which cells exit the cell cycle, it is particularly relevant to identify the cell cycle phase the cells in retentostat are in. In the current work population heterogeneity is addressed by monitoring the different, cell cycle-dependent, structures of actin. This study shows that, while retentostat cultures display many quiescence features, most cells (95-90%) remain in the active cell cycle and do not enter a quiescent phase. Combining these results with single-cell gene expression levels will bring insight in the regulatory mechanisms involved in the increased stress-resistance of retentostat cultures.

References

1) Boender LGM, et al. Appl. Environm. Microbiol. 2009;75:5607-5614.
2) Boender LGM, et al. BBA-Mol. Cell Res. 2011;1813: 2133-2144.
3) Boender LGM, et al. FEMS yeast research. 2011;11:603-620.

P142
AmdSYM, a new dominant recyclable marker cassette for *Saccharomyces cerevisiae*

D. Solis Escalante, G.A. Kuijpers, N. Bongaerts, I. Bolat, L. Bosman, J.T. Pronk, J.M.G. Daran, P. Daran-Lapujade
TU Delft, Biotechnology IMB-section, Delft

Despite the large collection of selectable marker genes available for *Saccharomyces cerevisiae*, marker availability can still present a hurdle when dozens of genetic manipulations are required. Recyclable markers, counterselectable cassettes that can be removed from the targeted genome after use, are therefore valuable assets in ambitious metabolic engineering programs. In the present work, the new recyclable dominant marker cassette amdSYM, formed by the Ashbya gossypii TEF2 promoter and terminator and a codon-optimized acetamidase gene (*Aspergillus nidulans* amdS), is presented. The amdSYM cassette confers *S. cerevisiae* the ability to use acetamide as sole nitrogen source. Direct repeats flanking the amdS gene allow for its efficient recombinative excision. As previously demonstrated in filamentous fungi, loss of the amdS marker cassette from *S. cerevisiae* can be rapidly selected for by growth in the presence of fluoroacetamide.

The amdSYM cassette can be used in different genetic backgrounds and represents the first counterselectable dominant marker gene cassette for use in *S. cerevisiae*. Furthermore, using astute cassette design, amdSYMexcision can be performed without leaving a scar or heterologous sequences in the targeted genome. The present work therefore demonstrates that amdSYM is a useful addition to the genetic engineering toolbox for *Saccharomyces* laboratory, wild, and industrial strains. This work was supported by the Netherlands Organisation for Scientific Research (NWO) and the Kluyver Centre for Genomics of Industrial Fermentation.

P143

A versatile, efficient strategy for assembly of multi-fragment expression vectors in *Saccharomyces cerevisiae* using 60 bp synthetic recombination sequences

N.G.A. Kuijpers, D. Solis Escalante, L. Bosman, M. van den Broek, J.T. Pronk, J.M. Daran, P. Daran-Lapujade
Delft University of Technology, Dept. of Biotechnology, Delft

In vivo recombination of overlapping DNA fragments for assembly of large DNA constructs in the yeast *Saccharomyces cerevisiae* holds great potential for pathway engineering on a small laboratory scale as well as for automated high-throughput strain construction. However, the current *in vivo* assembly methods are not consistent with respect to yields of correctly assembled constructs and standardization of parts required for routine laboratory implementation has not been explored. Here, we present and evaluate an optimized and robust method for *in vivo* assembly of plasmids from overlapping DNA fragments in *S. cerevisiae*.
To minimize occurrence of misassembled plasmids, two crucial improvements were introduced: (1) the essential elements of the vector backbone (yeast episome and selection marker) were disconnected and (2) standardized 60 bp synthetic recombination sequences non-homologous with the yeast genome were introduced at each flank of the assembly fragments. These modifications led to a 100 fold decrease in false positive transformants originating from the backbone as compared to previous methods. Implementation of the 60 bp synthetic recombination sequences enabled high flexibility in the design of complex expression constructs and allowed for fast and easy construction of all assembly fragments by PCR. The functionality of the method was demonstrated by the assembly of a 21 kb plasmid out of nine overlapping fragments carrying six glycolytic genes with a correct assembly yield of 95%. The assembled plasmid was shown to be a high fidelity replica of the *in silico* design and all glycolytic genes carried by the plasmid were proven to be fully functional.
The presented method delivers a substantial improvement for assembly of multi-fragment expression vectors in *S. cerevisiae*. Not only does it improve the efficiency of *in vivo* assembly of fragments in *S. cerevisiae*, but it also offers a versatile platform for easy and rapid design and assembly of synthetic constructs. The presented method is therefore ideally suited for the construction of complex pathways and for high throughput strain construction programs for metabolic engineering purposes. In addition its robustness and easy of use facilitates the construction of any plasmid carrying two genes or more.
This work was supported by the Kluyver Center for the Genomics of Industrial Fermentations and a vidi grant from the STW foundation

P144

Anaerobic cultivation of *Saccharomyces cerevisiae* under diurnal temperature cycles leads to cell cycle synchronization

M. Hebly, S. de Bruin, M.J.H. Almering, M.A.H. Luttik, J.T. Pronk, P. Daran-Lapujade
Delft University of Technology, Biotechnology, Delft

Temperature is known as a critical growth parameter for micro-organisms since changes in growth temperature will have a deep impact on the catalytic properties of all enzymes in metabolic networks. The overall aim of this study is to understand the kinetic and genetic regulatory strategies applied by *Saccharomyces cerevisiae* during evolution to adapt to diurnal temperature cycles. Therefore a yeast strain cultivated in an anaerobic glucose-limited chemostat was subjected to a dynamic temperature profile, in which the temperature was shifted from 30 °C to 12 °C and back (cycle length of 24 hours).
Budding index determination suggests that a substantial fraction of the cells (ca. 40-70%) synchronously divide during temperature cycles. This synchronization may result from the chosen growth rate (0.03 h^{-1}) for which the generation time of 23.1 hours is close to the duration of a temperature cycle (24 hours).
In this study we tried to elucidate whether anaerobic cultivation of *S. cerevisiae* under diurnal temperature cycles indeed led to cell cycle synchronization. To this end a flow cytometry-based method was implemented to more accurately define the cell cycle phase distribution among the yeast population during temperature oscillations.[1] The population dynamics was furthermore investigated via genome-wide transcriptome analysis. Sets of genes with cell-cycle-dependent expression have been previously published[2,3] and were mined to illustrate the synchronized regulation of gene expression. Comparison of flow cytometric and transcriptome data confirmed the cell cycle-dependent expression of well-known cell cycle markers. For instance the expression of the B-type cyclin CLB5, a G_1-marker, reached its maximum expression when flow-cytometric analysis indicated that a substantial fraction of the cells reached G_1 phase.
During temperature oscillations, many genes were differentially expressed, in addition the yeast culture displayed metabolic modifications, such as a switch between synthesis and degradation of reserve carbohydrates (trehalose and glycogen). Combined with the current dataset, future experiments with various temperature cycle lengths should enable the dissection between temperature-specific and cell cycle-specific responses.

Acknowledgements
This project was carried out within the research programme of the Kluyver Centre for Genomics of Industrial Fermentation and the Netherlands Consortium for Systems Biology.

References

1. Porro D, et al. Glucose metabolism and cell size in continuous cultures of Saccharomyces cerevisiae. Fems Microbiology Letters. 2003;229: 165-71.
2. Spellman PT, et al. Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by micro-array hybridization. Molecular Biology of the Cell. 1998;9:3273-97.
3. Cho RJ, et al. A genome-wide transcriptional analysis of the mitotic cell cycle. Molecular Cell 2. 1998:65-73.

P145

A study of 3,5 year on the surveillance of surgical site infections after vascular surgery

A.A. Ramcharan[1,2], E.E.J. Smeets[1], M.M.J. Rouflart[1], F.H. van Tiel[1,2], C.A. Bruggeman[1,2], C.G.M.I. Baeten[3], S.O. Breukink[3], J.H.M. Tordoir[3], E.E. Stobberingh[1,2]
[1]*Maastricht University Medical Center, Dept. of Medical Microbiology, Maastricht,* [2]*Maastricht University, School for Public Health and Primary Care (CAPHRI), Maastricht,* [3]*Maastricht University Medical Center, Dept. of Surgery, Maastricht*

Introduction: Surgical site infection (SSI) is a serious complication after vascular surgery, with a frequency of SSI ranging from 1% to 43%. Many interventions and guidelines are developed to reduce SSIs. Since a shorter postoperative hospital stay has become common practice, an increased number of SSI is diagnosed after discharge. To support an optimal choice for antibiotic prophylaxis and to investigate the organisms involved in SSI, bacteriological cultures are important. The objective was to assess the incidence of SSI after vascular surgery during and after hospitalization, to evaluate the effect of a Dutch safety programme (VMS) on SSIs and to investigate the microbiology of SSIs.
Methods: We assessed the incidence of SSI from July 2008 until December 2011, according to the Center for Disease Control and Prevention criteria, before (pre-test) and after (post-test) implementation of the VMS programme. Data was obtained during a thirty-day follow-up period after vascular surgery. SSI in relation to the wound class was assessed, as well as the microbiology of the wound swabs and the antibiotic susceptibility of the isolated micro-organisms. SSI rates were compared between the pre- and post-test, using the Pearson chi-square test.
Results: The study cohort comprised of 1719 surgeries. Of all 140 SSIs (8%) 39% was diagnosed after discharge. Most surgeries were classified as clean (82%). Risk factors for SSI during the whole study period were an emergency procedure and a contaminated or dirty wound class. The VMS programme resulted in a slight decrease in SSI. Of 38 (67%) wound swabs taken during hospitalization 66% yielded a positive bacteriological result. *Pseudomonas aeruginosa* (38%) was most frequently found, followed by *Staphylococcus aureus* (21%). The susceptibility of *P. aeruginosa* ranged from 73.5% for ciprofloxacin to 88% and 97% for ceftazidime and gentamicin, respectively. All *S. aureus* were susceptible for flucloxacillin, the macrolides, tetracycline, gentamicin and cefazolin. No methicillin-resistant *S. aureus* was found.
Conclusion: Postdischarge surveillance is essential for a reliable assessment of SSIs. Implementation of the VMS safety programme was only modestly successful so far; a larger period of time for implementation is necessary. Despite this, infection rates slightly decreased over time. Finally, microbiological culture of wounds is essential to know the causative micro-organisms including the antibiotic susceptibility, especially since antimicrobial resistance is increasing.

P146

Kingella kingae is an important cause of septic arthritis in children < 4 years of age

J. Heidema[1], B.J.M. Vlaminckx[2], E.J. van Hannen[2]
[1]*St Antoniusziekenhuis, Dept. of Paediatrics, Nieuwegein,* [2]*St Antoniusziekenhuis, Dept. of Medical Microbiology and Immunology, Nieuwegein*

Introduction: The correct treatment of acute osteo-articuar infections (AOI) in children is a matter of emergency because of its effect on bone development and growth. The last few years *Kingella kingae* has been recognized as an important pathogen in AOI in young children in several countries, but data from the Netherlands are missing. We here present the first data on the significance of *K. kingae* as a cause of culture negative septic arthritis in children in the Netherlands.

Methods: Children between 0-18 years of age with culture negative septic arthritis who underwent a synovial biopsy were included. Real time PCR was used to detect *K. kingae*.
Results: Synovial samples of 13 children that were culture negative after three days were collected. In seven samples *K. kingae* was detected. This was confirmed with 16S ribosomal PCR. In children with a negative *K. kingae* PCR, 1 child's enriched culture became positive for *Staphylococcus aureus* and in one child *Borrelia burgdorferi* was found. In four children no organism was found, but two of them developed the clinical picture of juvenile arthritis.
All children with a positive *K. kingae* PCR were 12 months to 4 years old. The four children with an unknown pathogen were between six weeks and ten years of age. All children with a positive *K. kingae* PCR had a mild and uncomplicated clinical course.
Conclusion: *K. kingae* is an important cause of septic arthritis in the Netherlands in children < 4 years of age. Because of its intrinsic resistance to some of the standard antimicrobials used in the treatment of AOI, diagnosing this pathogen is of utmost importance.

P147

Evaluation of an automated chemiluminescence immunoassay for detecting mumps immunoglobulin M (IgM) and IgG antibodies

J. Drenth, H. Feikens, C.A. Benne
Laboratory for Infectious Diseases, Groningen

Introduction: Recently, a chemiluminescence immunoassay for detecting mumps IgM has been introduced for the Liaison (Diasorin) platform in addition to the existing mumps IgG assay. We have evaluated the Liaison mumps IgM and IgG assay in comparison to a home made mumps IgM indirect immunofluorescence assay (IFA) and the Vidas (Biomérieux) mumps IgG enzyme linked fluorescent assay (ELFA).
Methods: For evaluation of the Liaison mumps IgM following serum samples previously tested for mumps IgM IFA were selected: 30 negative samples (titer < 32; 3 Vidas IgG negative and 1 indeterminate), 27 indeterminate samples (titer 32-64; 1 Vidas IgG negative and 1 indeterminate), 29 positive samples (titer ≥ 125; 1 Vidas IgG indeterminate). As far as possible, mumps IgM samples were chosen from patients that were also tested for mumps with PCR. 17 of the IFA IgM negatives were PCR negative and 9 were PCR positive. Of the IFA IgM indeterminates 2 were PCR positive and of the IFA IgM positives 1 was PCR negative and 2 were positive. For evaluation of the Liaison mumps IgG following serum samples previously tested in the Vidas ELFA for mumps IgG were selected: 20 negative samples (threshold value (TV) 0.06-0.34), 15 indeterminate samples (TV 0.36-0.49) and 27 positive samples (TV 0.50-6.61). Discrepant IgM and IgG samples were resolved with a mumps IgM and IgG enzyme immunoassay (Enzygnost, Siemens). True positive and true negative samples were defined as 2 out of 3 assays positive and negative, respectively.
Results: The Liaison IgM showed sensitivities and specificities of 80.6% and 87.7%, and 97.5% and 71% for indeterminates negative and positive, respectively. The sensitivities and specificities for the Liaison IgG were 100% and 88.2%, and 74.2% and 95.8% for indeterminates negative and positive, respectively. In comparison, the sensitivities and specificities of the IFA IgM were 93.5% and 100%, and 96.5% and 71.4% for indeterminates negative and positive, respectively. The sensitivities and specificities of the Vidas IgG were 100% and 100% for indeterminates negative and 100% and 83% for indeterminates positive. The intra assay coefficients of variation (CV) for the Liaison were 7.4% and 3.9% for IgM and IgG negative and 12.5% and 4.0% for IgM and IgG positive. The inter assay CV's for the Liaison were 4,5% and 9.4% for IgM and IgG negative and 12.8% and 6.4% for IgM and IgG positive.
Conclusion: The Liaison showed excellent reproducibility. With regard to the sensitivity and specificity the Liaison IgM and IgG assay performed somewhat less than the IFA IgM and Vidas IgG assays. The performance of the Liaison might have been influenced by sample selection and the high level of mumps vaccination or past infection in the studied population. Mumps re-infection does not always induce an IgM response as demonstrated by the high number of PCR positive results in IFA IgM negative samples. However, the automated design of both IgM and IgG assays can shorten the laboratory turn around time and save personal costs.

P148

Role of plasmids in the increased virulence of Dutch *Coxiella burnetii* goat strains

R. Kuley[1,2], H. Smith[1], F. Harders[1], J. Wells[2], M. Smits[1,2], D. Frangoulidis[3], H.J. Roest[1], A. Bossers[1]
[1]*Central Veterinary Institute of Wageningen UR, Wageningen,* [2]*Host Microbe Interactomics, Wageningen University, Wageningen,* [3]*Bundeswehr Institute of Microbiology, Munich, Germany*

Coxiella burnetii (Cb), the etiological zoonotic agent of 'Q fever' in humans and animals is an obligate intercellular BSL-3 bacterial pathogen. During 2007-2010 an unprecedented Q fever epidemic occurred in The Netherlands where over 4,000 human cases were registered but most likely over 40,000 people were infected. Molecular characterization of these unique Dutch outbreak isolates is essential for (molecular)epidemiological and vaccine development studies. Cb always carries a plasmid or plasmid-related DNA sequences in the chromosome, implying a critical function for some core plasmid genes. It is hypothesized that extra chromosomal sequences in Dutch Cb isolates could encode essential virulence modulators.
In this study the bacterial plasmids were isolated from Dutch Cb isolates and in all isolates we found plasmid or plasmid-derived sequences. The plasmids or plasmid-derived sequences in all Dutch goat strains were further identified by paired-end sequencing. We have demonstrated that Dutch Cb derived DNA sequences can increase the virulence of less virulent strains after transformation of plasmid or plasmid-derived sequences in a mouse virulence bioassay. Further investigation towards the mechanisms involved in the virulence and nature of these plasmid/plasmid-derived sequences in these Dutch strains as well as their interaction with components from the chromosome are essential to find the key virulence factors involved.

AUTHORS INDEX

Notities