nederlandse vereniging voor Medische Microbiologie

NEDERLANDS TIJDSCHRIFT VOOR MEDISCHE MICROBIOLOGIE

Supplement bij twintigste jaargang, april 2012

Voorjaarsvergadering van de Nederlandse Vereniging voor Medische Microbiologie (NVMM)
en de Koninklijke Nederlandse Vereniging voor Microbiologie (KNVM)

Papendal, 17 & 18 april 2012
Programma-overzicht
Abstracts
Auteursindex

20e jaargang . april 2012 . Supplement

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

NWO
Netherlands Organisation for Scientific Research

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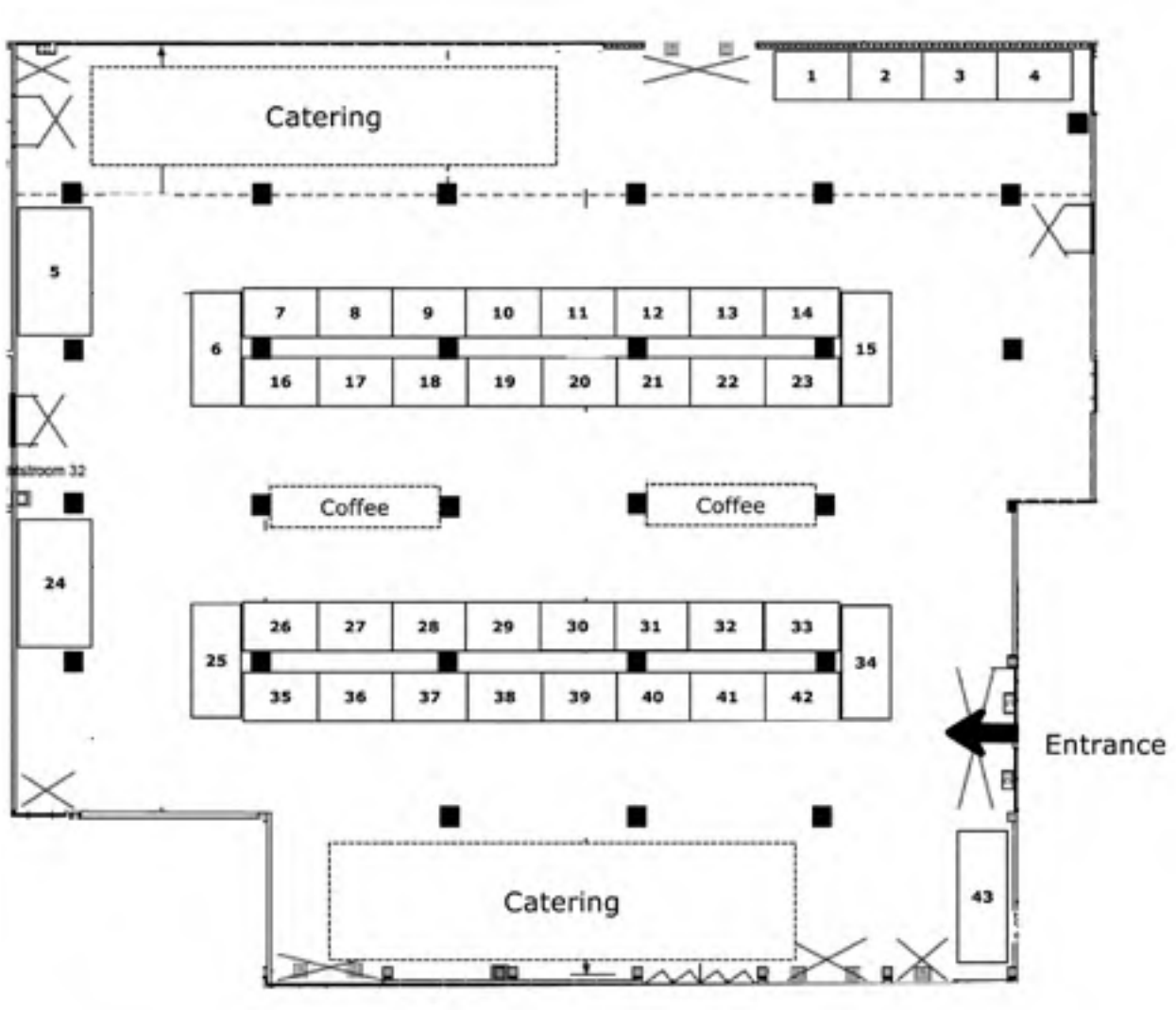

Booth number	Company
1	Mediaproducts
2	Elitech Benelux
3	Boom
6	DiaSorin
7	Gilead Sciences
8	Biolegio
10	Kiestra Lab Automation
11	Astellas Pharma NL
12	BaseClear
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15	Siemens Healthcare Diagnostics
16	MSD
17	MIPS
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25	Bio Rad Laboratories
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33	Oxoid
34	Minigrip Nederland
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37	Clindia Benelux
38	Mediphos Medical Supplies
39	R-Biopharm
40	BioMerieux Benelux
41	Sarstedt
42	Bio Trading Benelux
43	Roche Diagnostics

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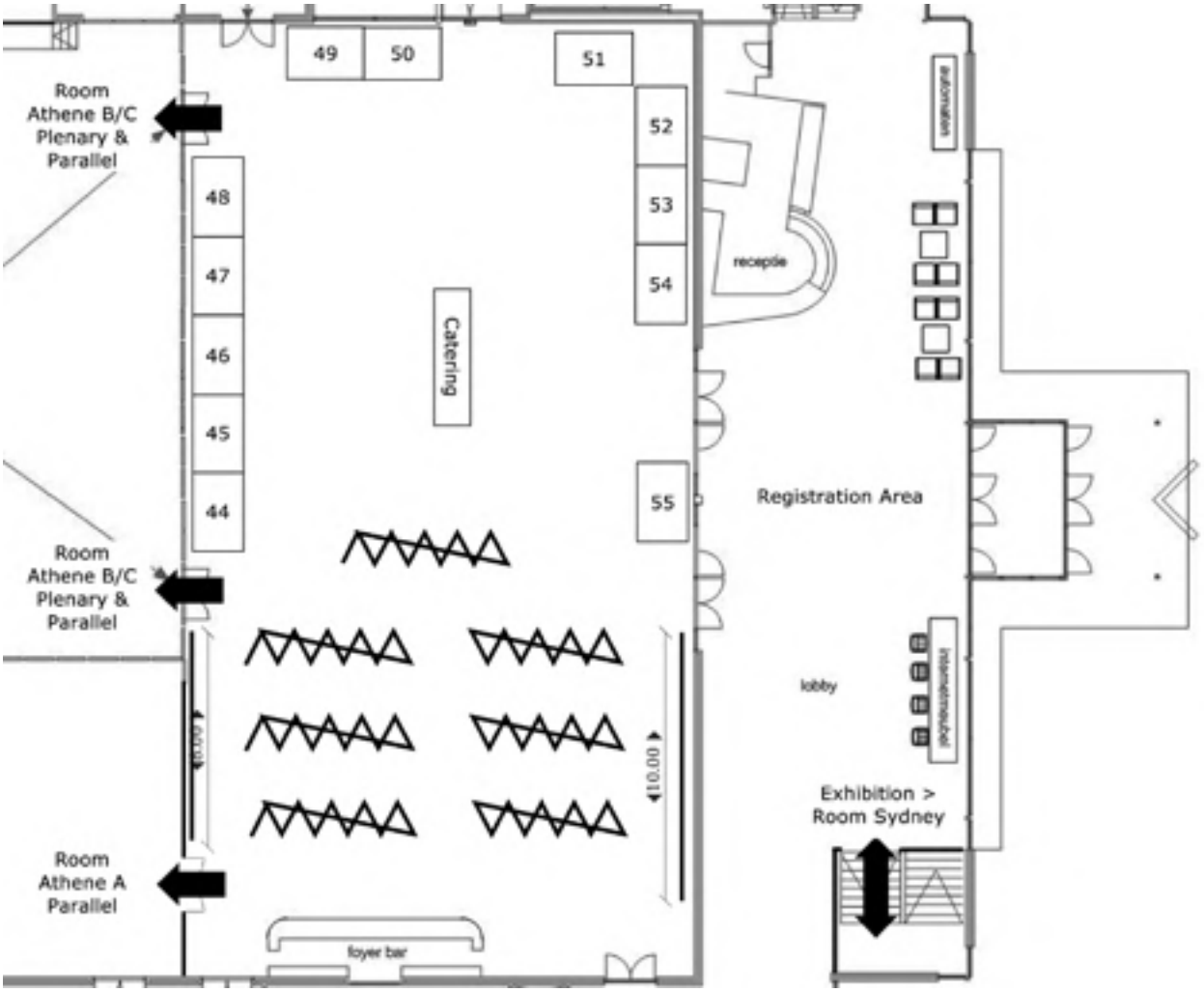

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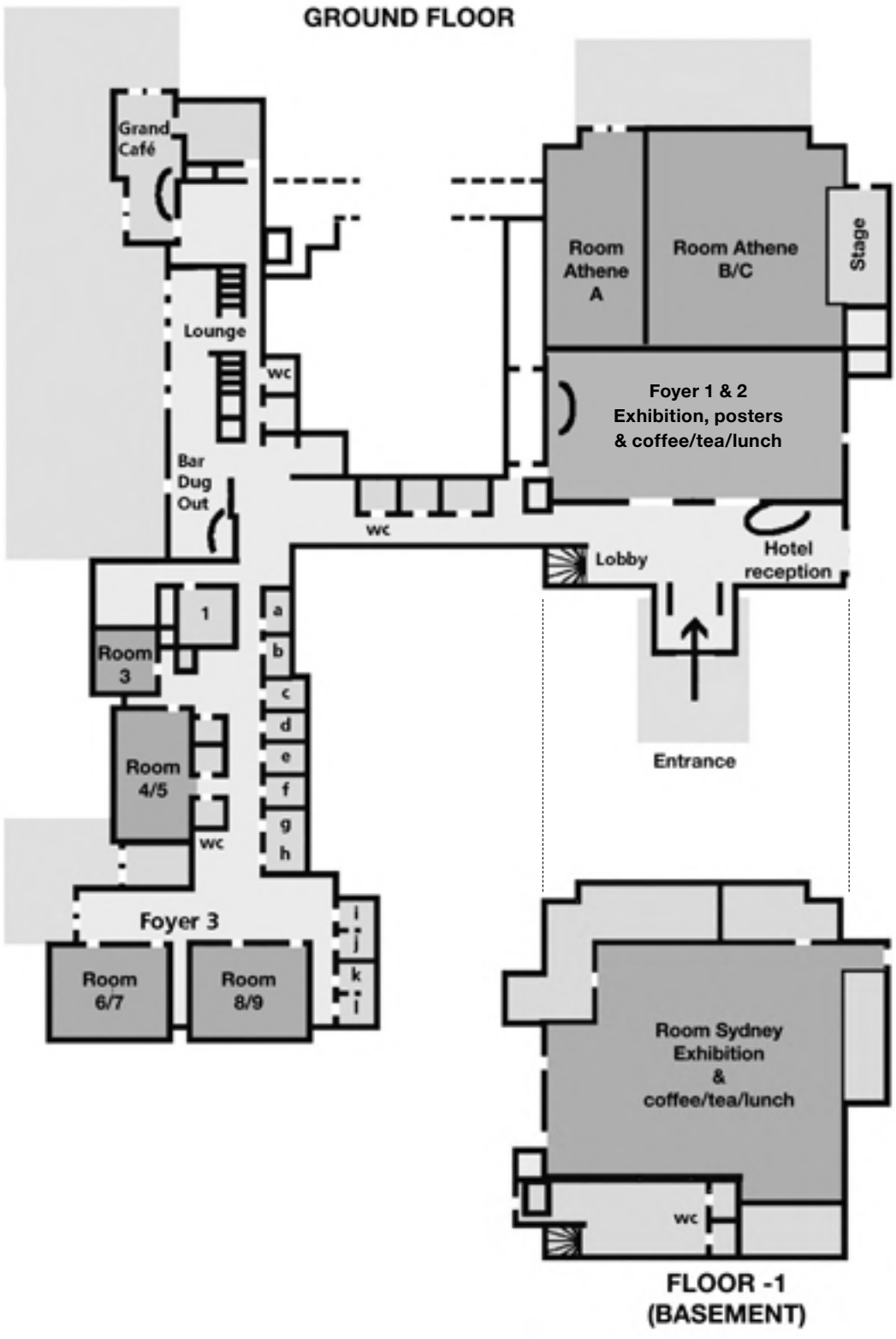
GROUND FLOOR
Grand Café
Lounge
wc
Bar
Dug
Out
Room Athene A
Room Athene B/C
Stage
Foyer 1 & 2
Exhibition, posters
& coffee/tea/lunch
wc
Lobby
Hotel reception
Entrance
1
Room 3
a
b
c
d
e
f
g
h
Room 4/5
wc
Foyer 3
i
j
k
l
Room 6/7
Room 8/9
Room Sydney
Exhibition
&
coffee/tea/lunch
wc
FLOOR -1
(BASEMENT)

TUESDAY APRIL 17 2012

09:00 - 09:30 **Registration**

Athene B/C

09:30 - 11:00 **Plenary session**

Chair: W. Bitter

09:30 - 10:15 **Complex dynamics in microbial communities**

O001 J. Huisman (The Netherlands)

10:15 - 11:00 **Electromicrobiology**

O002 D.R. Lovley (USA)

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

Athene B/C

11:30 - 13:30 **Plenary session & award ceremony**

Chair: B.J.M. Vlaminckx

11:30 - 12:15 **The curious road from poxvirus tropism to oncolytic virotherapy**

O003 G. McFadden (USA)

12:15 - 13:00 **Sustainability of antibiotic efficacy: from containment to restorative approaches**

O004 F. Baquero (Spain)

13:00 - 13:30 **Award ceremony**

13:30 - 14:30 **Lunch**

Athene B/C

13:30 - 14:30 **Lunch Symposium Diasorin: The new Liaison® XL Murex Hepatitis and Retrovirus Assays: experiences from the field**

Chair: Dr. Ph.H. Rothbart
Dr. M. Schutten, Erasmus MC, Rotterdam
Dr. C.F.M. Linssen, Atrium Medisch Centrum, Heerlen

Athene A

13:30 - 14:30 **KNVM Business meeting**

14:30 - 16:00 **Parallel sessions**

Athene B/C **From STEC to HUSEC – EHEC yesterday and today**

Chair: R.A. Coutinho

14:30 - 15:00 **EHEC O104:H4: Phylogenetic origin and pathogenesis**

O005 A. Mellmann (Germany)

15:00 - 15:30 **Molecular epidemiology and clinical risk assessment of Shiga-producing *E. coli* (STEC)**

O006 A.W. Friedrich

15:30 - 15:45 **Subtractive epidemiology of ESBL producing Enterobacteriaceae in the northern Dutch-German border region**

O007 S. Surie

15:45 - 16:00 **High acquisition rates of ESBL producing Enterobacteriaceae among Dutch travelers**

O008 S. Paltansing

Athene A **West Nile virus emergence in Europe**

Chairs: C. Reusken and M. Koopmans

14:30 - 15:00 **Emergence of West Nile virus in Greece**

O009 A. Papa (Greece)

15:00 - 15:30 **West Nile virus non-coding RNA: effects on pathogenicity and mosquito transmission**

O010 G.P. Pijlman

15:30 - 15:45 **The role of immature virus particles in Dengue pathogenesis**

O011 J.M. da Silva Voorham

15:45 - 16:00 **Detection of Dengue NS1 in travelers: a comparison of the performance of three rapid diagnostic tests with the Platelia NS1 antigen ELISA**

O012 C. Liem

Room 3 **Surprising infectious diseases and association with pathogens**

Chair: E.J. Kuijper

14:30 - 14:45 **An outbreak of histoplasmosis misdiagnosed as miliary tuberculosis among participants of a tropical biology course in Uganda**

O013 M.A. Schouten

14:45 - 15:00 **Outbreak of a multi-drug resistant *Pseudomonas aeruginosa* on the intensive care unit of a tertiary care hospital in the Netherlands; a case-control study to identify sources and risk factors**

O014 M. Knoester

15:00 - 15:15 **The curious case of a man who slept in the daytime**

O015 M.B.B. McCall

15:15 - 15:30 **Appendiceal spirochaetosis in children**

O016 L.J. Westerman

15:30 - 15:45 **Asymptomatic carriage of *Mycoplasma pneumoniae* in the upper respiratory tract of children**

O017 C. Vink

15:45 - 16:00 **Pneumococcal pneumonia: the association between clinical severity and serotype**

O018 A.J.H. Cremers

Room 4/5 **Multiresistent microorganisms in hospitals: recognizing outbreaks, management and responsibilities**

Chair: M. Vos

14:30 - 15:00 **Recognizing outbreaks: pitfalls in diagnosing carbapenemase producing microorganisms**

O019 T. van Ossewaarde

15:00 - 15:15 **Management of outbreaks: guidelines and responsibilities – WIP guidelines on multi-resistant microorganisms**

O020 I.J.B. Spijkerman

15:15 - 15:30 **Management of outbreaks: guidelines and responsibilities – Responsibilities in an outbreak situation**

O021 G. Haringhuizen

15:30 - 15:45 **Management of outbreaks: guidelines and responsibilities – SWAB guidelines on the treatment of multiresistant microorganisms**

O022 J.W. Mouton

15:45 - 16:00 **National surveillance of outbreaks**

O023 C.H.E. Boel

Room 6/7 **Role of oxygen in lifestyle choices of microbial pathogens**

Chairs: J. Stoof and A. van Vliet

14:30 - 15:00 **Bacterial oxygen sensing: lessons taught by *Escherichia coli***

O024 J. Green (United Kingdom)

15:00 - 15:30 **General and oxidative stress responses in foodborne pathogens**

O025 T. Abee

15:30 - 15:45 **Translational regulation of the respiratory electron transport chain of *Neisseria meningitidis* by the Fur controlled small non-coding RNA NrrF**

O026 Y. Pannekoek

15:45 - 16:00 **Genome-wide identification of *Streptococcus pneumoniae* genes essential for growth and survival in CO_2-poor environmental conditions**

O027 P.J. Burghout

Room 8/9 **Photosynthetic microbes: ecophysiology and applications**

Chairs: M. Al-Najjar and M. Kühl

14:30 - 15:00 **Ecology of chlorophyll-d containing cyanobacteria**

O028 M. Kühl (Denmark)

15:00 - 15:30 **Redirecting the intermediary metabolism of *Synechocystis* PCC6803 for sustainable biofuel production**

O029 K.J. Hellingwerf

15:30 - 15:45 **Energy budget and light utilization efficiency in photosynthetic microbial mats**

O030 M. Al-Najjar (Germany)

15:45 - 16:00 **Transition between oxygenic and anoxygenic photosynthesis in cyanobacteria from the Frasassi sulfidic springs**

O031 J.M.K. Klatt (Germany)

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

16:30 - 18:00 **Parallel sessions**

Athene B/C **Psittacosis**

Chairs: B. Mulder and W. Dorigo

16:30 - 17:00 **Microbiology and pathogenesis of psittacosis**

O032 D. Vanrompay (Belgium)

17:00 - 17:15 **Diagnostics and typing of psittacosis**

O033 E. Heddema

17:15 - 17:30 **Veterinary aspects and control of psittacosis**

O034 O.F.J. Stenvers

17:30 - 17:45 **Multi locus sequence typing of *Chlamydia psittaci* and *Chlamydia pecorum* from birds and mammals reveals an association between Chlamydia genotypes and host species**

O035 Y. Pannekoek

17:45 - 18:00 **The challenges of serological prediction of chronic Q fever**

O036 L.M. Kampschreur

Athene A **Mycology**

Chairs: H.A.B. Wösten and P. Verweij

16:30 - 16:45 **Discovery of a new azole resistance mechanism in *Aspergillus fumigatus* through whole genome sequencing and sexual crossing**

O037 S.M.T. Camps

16:45 - 17:00 **Mannitol-1-phosphate dehydrogenase is essential for the development of extreme stress resistant fungal ascospores**

O038 T.T. Wyatt

17:00 - 17:15 **Effects of corticosteroids on submerged growth of *Aspergillus fumigatus* and *Aspergillus niger***

O039 E. Bathoorn

17:15 - 17:30 **Identification of moulds with Matrix Assisted Laser Desorption and Ionization- Time of Flight Mass Spectrometry**

O040 A.L. Klink

17:30 - 17:45 ***Aspergillus fumigatus* mycovirus infection is not dependent on the genetic up-make of the host**

O041 J.M. Refos

17:45 - 18:00 **Filamentous fungi in cystic fibrosis patients in the Netherlands**

O042 P.D. Terpstra

Room 3 **Virology**

Chair: H.G.M. Niesters

16:30 - 16:45 **Active polyomavirus infection characterizes trichodysplasia spinulosa**

O043 M.C.W. Feltkamp

16:45 - 17:00 **The paradox of maternal immunity as a risk factor for congenital cytomegalovirus infection: a population-based prediction model**

O044 J.C. de Vries

17:00 - 17:15 **Binding of avian coronavirus spike proteins to host factors reflects virus tropism and pathogenicity**

O045 M.H. Verheije

17:15 - 17:30 **The use of MMP-8 and MMP-9 to assess disease severity in children with viral lower respiratory tract infections**

O046 G. Ferwerda

17:30 - 17:45 **Norovirus in hospitalized children: clinical, epidemiological and virological features**

O047 J.C. Rahamat-Langendoen

17:45 - 18:00 **Stimulation of TLR3 or TLR9 on dendritic cells and fibroblasts limits herpes simplex virus type 1 infection in an IFNß-dependent and –independent way**

O048 R.J.L. Gaajetaan

Room 4/5 **Mycobacterial disease: new insights to improve treatment and outcome**

Chairs: J. van Ingen and B. Mulder

16:30 - 17:00 **New insights question the current rifampicin dose in tuberculosis treatment**

O049 J.E.M. de Steenwinkel

17:00 - 17:30 **Pharmacokinetics of nontuberculous mycobacterial disease treatment regimens**

O050 J. van Ingen

17:30 - 17:45 **Minimum inhibition concentration of first and second line drugs against *Mycobacterium tuberculosis* complex isolates in relation to genetic mutations**

O051 G. van der Laan

17:45 - 18:00 **Energy metabolism, diarylquinolines and pyrazinamide: insight in the molecular mechanism of drugs that may shorten tuberculosis treatment**

O052 D. Bald

Room 6/7 **Outer membrane proteins of Gram negative bacteria and Mycobacteria, similarities and differences**

Chair: E.N.G. Houben

16:30 - 17:00 **Outer membrane proteins in mycobacteria**

O053 M. Niederweis (USA)

17:00 - 17:30 **Outer membrane proteins in Gram-negative bacteria: structure, function and biogenesis**

O054 J.P.M. Tommassen

17:30 - 17:45 **Differential detergent extraction of *Mycobacterium marinum* cell envelopes reveals novel, extensively modified, outer membrane protein**

O055 A.D. van der Woude

17:45 - 18:00 **Omp32 of *Helicobacter pylori* is involved in nickel and cobalt transport**

O056 J. Stoof (United Kingdom)

Room 8/9 **Physiology & system biology**

Chairs: S. Brul and M.H. Zwietering

16:30 - 16:45 **Detection of genes essential for growth of respiratory pathogens**

O057 A. Zomer

16:45 - 17:00 **Unraveling the small regulatory RNA network of *Bacillus subtilis***

O058 R.A.T. Mars

17:00 - 17:15 **From transcriptional landscapes to prediction of stress induced robustness using biomarkers**

O059 H.M.W. den Besten

17:15 - 17:30 **Characterisation of the biodiversity of spoilage *Lactobacilli***

O060 J.W. Sanders

17:30 - 17:45 **Protein complexes involved in the electron transport chain of anammox bacteria**

O061 N.M. de Almeida

17:45 - 18:00 **A multi-platform flow device for microbial cultivation and microscopic analysis**

O062 B.M. Ryback

Room Sydney

18:00 - 18:30 **Drinks**

Restaurant

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

Foyer 1 & 2

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

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

Athene A

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

WEDNESDAY 18 APRIL 2012

08:30 - 09:00 **Registration**

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

Athene B/C **Multidrug resistance: ESBL and carbapenemase**

Chair: J.W.T. Cohen Stuart

09:00 - 09:15 **A case of New Delhi metallo-beta-lactamase 1 (NDM-1) in the Netherlands with secondary transmission**

O063 T. Halaby

09:15 - 09:30 **Prevalence of rectal carriage of extended-spectrum beta-lactamase producing Enterobacteriaceae in hospitalised patients: 2010 and 2011**

O064 I. Willemsen

09:30 - 09:45 **Optimizing the Dutch carbapenemase detection guideline for OXA-48 producing *E. coli* associated with a large outbreak**

O065 J.W.T. Cohen Stuart

09:45 - 10:00 **Surveillance of carbapenemase producing Enterobacteriaceae in The Netherlands**

O066 D.W. Notermans

10:00 - 10:15 **The impact of clinical breakpoint changes on surveillance of antimicrobial resistance in Enterobacteriaceae causing bacteraemia**

O067 A.K. van der Bij

10:15 - 10:30 **Cefotaxime resistant Enterobacteriaceae in fecal samples of dogs and cats**

O068 A. Schoormans

Athene A **Vaccines are not forever**

Chairs: P.W.M. Hermans and F.R. Mooi

09:00 - 09:30 **Towards predicting the antigenic evolution of influenza virus**

O069 R.A.M. Fouchier

09:30 - 09:45 **Changes in the composition of the pneumococcal population in the Netherlands after the implementation of the 7-valent pneumococcal vaccine**

O070 K.E.M. Elberse

09:45 - 10:00 **Mucosal immunization protects mice against influenza virus-induced pneumococcal otitis media**

O071 D.A. Diavatopoulos

10:00 - 10:15 **Phylogeny of European *Bordetella pertussis* and the occurrence of vaccine antigen deficient (VAD) mutants**

O072 A. Zeddeman

10:15 - 10:30 **In search for novel pertussis vaccine targets: a comprehensive transcriptomic and proteomic approach**

O073 D. Gouw

Room 3 **Asplenia: infection and prevention**

Chair: R.W. Vreede

09:00 - 09:30 **Life-threatening infections due to asplenia**

O074 F.P. Kroon

09:30 - 10:00 **A new LCI guideline for prevention of infections in patients with hypo- and asplenia**

O075 A.J.J. Lammers

10:00 - 10:30 **Efficacy of vaccinations after splenectomy**

O076 A. Meerveld-Eggink

Room 4/5 **WMDI: Human genetic factors in relation to microbial infection**

Chairs: E.C.J. Claas and J.W.A. Rossen

09:00 - 09:30 **Human genetic susceptibility to bacterial infections**

O077 J.T. van Dissel

09:30 - 10:00 **Genetic susceptibility to viral Lower Respiratory Tract Infections (LRTI) in Europe**

O078 A. Rautanen (United Kingdom)

10:00 - 10:30 **Genetic predisposition to chronic mucocutaneous candidiasis**

O079 F. van de Veerdonk

Room 6/7 **Gene regulation via alternative sigma factors and their role in virulence**

Chair: W. Bitter

09:00 - 09:30 **Role of alternative sigma factors of *Mycobacterium tuberculosis* in virulence**

O081 R. Manganelli (Italy)

09:30 - 10:00 **Alternative sigma factor regulation in pseudomonas**

O082 M. Llamas (Spain)

10:00 - 10:15 **Promoter propagation in prokaryotes**

O083 M.W.J. van Passel

10:15 - 10:30 **A novel cell-surface signalling system uncovers the intimate relationships between the components of this signalling cascade**

O084 K.C.J.T. Bastiaansen

Room 8/9 **Differentiation in microbial multicellular communities**

Chair: D. Claessen

09:00 - 09:30 **Differentiation in multicellular *Bacillus subtilis* communities**

O085 D. López (Germany)

09:30 - 10:00 **Heterogeneity in the fungal mycelium**

O086 H.A.B. Wösten

10:00 - 10:15 **Common genes are required for architecturally complex colony formation and sliding motility of *Bacillus subtilis***

O087 A.T. Kovacs

10:15 - 10:30 **Spatially resolving the secretome within the mycelium of the cell factory *Aspergillus niger***

O088 P. Krijgsheld

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

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

Athene B/C **Microbial education & social media**

Chair: A.J.W. van Alphen

11:00 - 11:30 **Social media and microbial education**

O089 A.J. Cann (United Kingdom)

11:30 - 11:45 **Distant learning**

O090 M. Reij

11:45 - 12:00 **Social media and infection control: trending topic?**

O091 L.S. van Velsen

12:00 - 12:15 **Post-doctoral e-learning for professionals in medical microbiology**

O092 J.W. Mouton

12:15 - 12:30 **Blended learning in a laboratory school**

O093 E.M. van Hove

Athene A **Kingdomcrossers, pathogens hopping between plant, animal and humans**

Chairs: J. van Doorn and P.H.M. Savelkoul

11:00 - 11:30 **We only find what we look for: can enteric pathogens of animals live on and be spread by plants?**

O094 I.K. Toth (United Kingdom)

11:30 - 12:00 **Barriers and bypasses to lateral gene transfer in prokaryotes**

O095 T.A.L. Dagan (Germany)

12:00 - 12:15 **Zoo animal gut microbiota as a reservoir of antibiotic resistance genes**

O096 T.D.J. Bello Gonzalez

12:15 - 12:30 **Functional metagenomic analysis reveals selection for antibiotic resistance in the gut microbiota during Intensive Care hospitalization**

O097 E.B. Buelow

Room 3 **Streptococci**

Chairs: S.H.M. Rooijakkers and H.J. Bootsma

11:00 - 11:15 **Genetic identification and virulence contribution of the Group A streptococcal antigen**

O098 N.M. van Sorge

11:15 - 11:30 **A *Streptococcus pneumoniae* operon modifying interaction with host cells in a capsule-dependent manner**

O099 J.J.E. Bijlsma

11:30 - 11:45 **Pneumococcal immune evasion by Zinc metalloprotease C**

O100 G.J. Surewaard

11:45 - 12:00 **Interaction between *Streptococcus pneumoniae*, receptors and brain endothelial cells in an experimental meningitis model**

O101 F. Iovino

12:00 - 12:15 **Identification of novel pneumococcal adherence factors by a combination of genome-wide approaches**

O102 H.J. Bootsma

12:15 - 12:30 **Membrane attack complex deposition on Gram-positive bacteria**

O103 E.T.M. Berends

Room 4/5 **Lyme arthritis and neuroborreliosis: clinical recognition and laboratory tests**

Chairs: A. van Dam and E.J. Kuijper

11:00 - 11:30 **CXCL13 as a diagnostic marker for Lyme neuroborreliosis**

O104 T. Rupprecht (Germany)

11:30 - 11:45 **Testing for Lyme Disease: when and how?**

O105 N. van Burgel

11:45 - 12:00 **Lyme arthritis; clinical diagnosis or only laboratory confirmed?**

O106 A. Brandenburg

12:00 - 12:15 **Comparison of the inter-laboratory performance of Lyme disease serology in the Netherlands by quality assessment**

O107 T. Herremans

12:15 - 12:30 **Performance of five VlsE containing immunoassays for the diagnosis of Lyme borreliosis**

O108 A.P. van Dam

Room 6/7 **Type V secretion: mechanism and use in vaccine development**

Chair: S. Luirink

11:00 - 11:30 **A generalised module for the selective extracellular accumulation of recombinant proteins**

O109 I.R. Henderson (United Kingdom)

11:30 - 12:00 **Structure and function of type Vc and type Ve autotransporters**

O110 D. Linke (Germany)

12:00 - 12:15 **Versatile microbial Autotransporter platforms for the display and secretion of multiple heterologous proteins**

O111 W.S.P. Jong

12:15 - 12:30 **Specificity of target selection by the TpsB outer membrane transporters of co-existing Two Partner Secretion systems of the human pathogen *Neisseria meningitidis***

O112 S. Rahman

Room 8/9 **Robustness of complex microbial communities: implications for microbial pathology and food fermentations**

Chairs: D. Budding and E.J. Smid

11:00 - 11:30 **Studying the gut microbiota using metagenomics**

O113 J. Raes

11:30 - 12:00 **Multilevel approach reveals high level of clonal and functional diversity in complex dairy starter culture**

O114 O. Erkus

12:00 - 12:15 **Population dynamics in an undefined mixed starter culture - the role of bacteriophages**

O115 M. Spus

12:15 - 12:30 **Immune evasion dynamics during *Staphylococcus aureus* biofilm growth**

O116 R. Nijland

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

Athene A

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

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

Athene B/C **Microbial pathogenesis**

Chairs: J.J.E. Bijlsma and N.M. van Sorge

14:00 - 14:15 **Modulated lipooligosaccharide structure prevents *Haemophilus influenzae* from IgM-mediated complement killing during otitis media**

O117 J.D. Langereis

14:15 - 14:30 **Profiling the antibody response in acute and chronic patients from a recent Dutch Q fever outbreak by protein microarray**

O118 T. Herremans

14:30 - 14:45 **Secretome phage display library, filling the gap between genomic data and functional studies**

O119 P.J.A. Haas

14:45 - 15:00 **Induction of protective T-cell responses by *Aspergillus fumigatus* in humans**

O120 M.S. Gresnigt

15:00 - 15:15 **A general secretion signal for the type VII protein secretion pathway in pathogenic mycobacteria**

O121 W. Bitter

15:15 - 15:30 ***Staphylococcus aureus* secretes three Extracellular adherence proteins (Eap) that inhibit Neutrophil elastase**

O122 D.A.C. Stapels

Athene A **Into the deep: applications of next generation sequencing in clinical virology**

Chair: M. Koopmans and R. Schuurman

14:00 - 14:30 **Discovery of Schmallenberg virus**

O123 M. Beer (Germany)

14:30 - 14:45 **Longitudinal next generation "Deep" sequence analysis of Dual/Mixed HIV infected patients treated with Maraviroc demonstrates rapid selection for X4-predicted virus with extremely low FPR**

O124 J. Symons

14:45 - 15:00 **Evaluation of persistence of resistant variants with ultra-deep pyrosequencing in chronic Hepatitis C patients treated with Telaprevir**

O125 X.V. Thomas

15:00 - 15:15 **HIV-1 Reverse Transcriptase drug resistance mutations M184V, M184I and M184T have a differential impact on Entecavir incorporation and susceptibility**

O126 M. Nijhuis

15:15 - 15:30 **Comparison between Sanger cloning sequencing and ultra-deep sequencing techniques for the characterization of Hepatitis C quasispecies**

O127 C.K.Y. Ho

Room 3 **STI treatment failure: the need for early diagnosis!**

Chair: H.J. Thjie and H. de Vries

14:00 - 14:30 **Challenges to the public health control of bacterial STIs, in particular gonorrhoea**

O128 C. Ison (United Kingdom)

14:30 - 15:00 **Questioning azitromycin for uncomplicated urogenital Chlamydia infection**

O129 M. Vandenbruaene (Belgium)

15:00 - 15:15 **Recent trends in gonococcal resistance against third-generation cephalosporins and azithromycin**

O130 A.P. van Dam

15:15 - 15:30 **Improved diagnostics of bacterial vaginosis with molecular techniques**

O131 A.G.C.L. Speksnijder

Room 4/5 **Clinical microbiology**

Chairs: B.J.M. Vlaminckx and M.J.H.M. Wolfhagen

14:00 - 14:15 **Outcome of nosocomial *Clostridium difficile* infections; results of a multicenter cohort study**

O132 M.P.M. Hensgens

14:15 - 14:30 **Prolonged bacterial culture to detect periprosthetic joint infection: how long is long enough?**

O133 K. Waar

14:30 - 14:45 **Evaluation of several biochemical and molecular techniques for identification of *Streptococcus pneumonia* and *Streptococcus pseudopneumoniae* and detection of these bacteria in respiratory samples**

O134 E. Wessels

14:45 - 15:00 **Routine identification of clinical isolates of anaerobic bacteria: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry performs better than conventional identification methods**

O135 M. Knoester

15:00 - 15:15 **Empiric therapy for sepsis needs adaptation due to increased resistance rates**

O136 M.A. Leverstein – van Hall

15:15 - 15:30 **Antimicrobial susceptibility testing in ninety minutes by bacterial cell count monitoring**

O137 N.L.A. Arents

Room 6/7 **CRISPR-Cas: more than just phage defence**

Chairs: P. van Baarlen and R. Louwen

14:00 - 14:30 **CRISPR: a small RNA guided immune system**

O138 S.J.J. Brouns

14:30 - 15:00 **crRNA maturation: a key event in the activation of the CRISPR/Cas immune system**

O139 E. Charpentier (Sweden)

15:00 - 15:15 **Transcription of *Campylobacter jejuni* CRISPR RNAs is initiated from a sigma70 promoter located in each separate CRISPR repeat**

O140 A.H.M. van Vliet (United Kingdom)

15:15 - 15:30 **CRISPR-Cas system in the genomes of *Campylobacter fetus* subspecies**

O141 B. Duim

Room 8/9 **Biomarkers for microbial robustness**

Chairs: T. Abee and H. den Besten

14:00 - 14:30 **Robustness of probiotics**

O142 P. Ross (Ireland)

14:30 - 15:00 **Enhanced functionality LAB starters and probiotics**

O143 M. Kleerebezem

15:00 - 15:30 **Bacterial spore stress resistance; a proteomics quest for bio-markers**

O144 S. Brul

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

Athene B/C

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

O001

Complex dynamics in microbial communities

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Many traditional studies of species interactions have focused on equilibrium dynamics. Examples in microbial ecology are provided by chemostat studies in which species interactions like competition and predation are investigated until steady state is reached. In this presentation, we will take a different perspective by highlighting the non-equilibrium dynamics of microbial communities.

First, we will show that multi-species communities can display non-equilibrium dynamics even in a constant environment. The interplay between multiple species may result in permanent changes in microbial communities, with continuous ups and downs in species abundances and species composition never reaching an equilibrium state. This is illustrated by controlled laboratory experiments demonstrating chaos in microbial food webs.

Next, we will investigate possible underlying mechanisms that may generate such complex dynamics. Networks of species interactions often contain oscillating sub-units, for instance predator and prey species that would display classical predator-prey oscillations if isolated from the rest of the community. Analysis of experimental data shows that the interplay between several oscillating sub-units (e.g., several predator-prey interactions within a larger network) can cause intriguing species fluctuations, in which the community shifts back and forth between different predator-prey cycles in a chaotic fashion.

Finally, we will consider the role of environmental variation, and show that minor environmental fluctuations can strongly amplify fluctuations in species abundances through a phenomenon known as resonance. The magnitude of resonance depends on the characteristic time scale of the environmental fluctuations in comparison to the intrinsic time scale of the species dynamics, which suggests that organisms may tune their life cycle to the time scales of environmental fluctuations. As an illustration, we show that the relatively fast temperature fluctuations in shallow lakes fall within the range to which fast-growing freshwater plankton are most sensitive, while slowly growing copepods and krill of marine ecosystems will tend to resonate more strongly with the slower temperature variability of the open ocean.

Microbial communities typically consist of numerous species, involved in a multitude of species interactions and embedded in variable environments. Hence, this non-equilibrium perspective on the species composition of microbial communities may find application in a wide range of different fields. Examples include studies of natural communities in terrestrial, freshwater and marine ecosystems, but also the microbial gut flora, microbial disease dynamics, or the use of microbial communities in wastewater treatment and other biotechnological applications.

O002

Electromicrobiology

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Electromicrobiology is a rapidly emerging field of study that investigates microbial electron exchange with electrodes and novel electronic properties of microorganisms that are of interest for developing electronic devices. Principles developed from electromicrobiology studies have also recently provided insight into the microbial ecology of natural environments.

Many of the advances in electromicrobiology have arisen from the study of microbial fuel cells. These are devices that were initially designed for harvesting electricity from organic matter, but have additional practical applications such as monitoring microbial activity and stimulating the degradation of organic contaminants in sedimentary environments. It is often found that the electrodes accepting electrons in microbial fuel cells are heavily colonized by *Geobacter* species.

Geobacter species produce the highest current densities of any known pure culture. They can completely oxidize organic compounds to carbon dioxide with direct electron transfer to electrodes. Thick (> 80 μm) biofilms form on the electrodes. Cells at substantial distance from the electrode remain metabolically active and contribute as much to current production as cells in close association with the electrode surface. This is possible because the *Geobacter* biofilms are highly conductive, with conductivities rivaling that of synthetic, conducting polymers. Significant quantities of the multi-heme, *c*-type cytochrome, OmcZ, accumulate at the biofilm/electrode interface and multiple lines of evidence suggest that OmcZ facilitates electron transfer from the conductive biofilm to the electrode.

The high conductivity of *Geobacter* biofilms can be attributed to long-range electron transport via a network of conductive pili coursing through the biofilm. These

pili, which are also known as microbial nanowires, exhibit metallic-like conductivity, a phenomenon that has not previously been observed in biological material, and is distinctly different than the short-range electron hopping or tunneling between two juxtapositioned molecules that typically characterize microbial electron transport. In addition to their high conductivity, the *Geobacter* biofilms can function as supercapacitors and transistors. With genetic engineering it was possible to modify conductivity and supercapacitor characteristics. Increasing conductivity of the biofilms increased their current-generating capacity. When electrodes are poised at a sufficiently low potential some microorganisms can directly accept electrons from electrodes to support anaerobic respiration. One of the most exciting practical applications of this phenomenon is microbial electrosynthesis, in which microbial biofilms on electrodes are provided electrons to reduce carbon dioxide to produce fuels or other organic commodities that are excreted from the cells. When microbial electrosynthesis is powered with solar technology it represents an artificial form of photosynthesis that is much more efficient than plant/algal photosynthesis in converting solar energy to desired products. In addition to its higher efficiency, microbial electrosynthesis is also more environmentally sustainable than biomass-based strategies because it does not require arable land, avoids environmental degradation associated with intensive agriculture, and requires much less water.

A spin-off of studies on microbe-electrode interactions has been the discovery that some microorganisms can make direct cell-to-cell electrical connections via mechanisms that appear to be similar to interactions with electrodes. For example, natural aggregates from methanogenic wastewater digesters have metallic-like conductivity and multiple lines of evidence suggest that methanogens primarily receive electrons directly rather than via interspecies hydrogen transfer.

O003

The curious road from poxvirus tropism to oncolytic virotherapy

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Myxoma virus (MYXV) is a leporipoxvirus that causes an acute lethal infection only in European rabbits (*Orcytolagus cuniculis*), but in evolutionary terms the virus co-evolved within American lagomorphs of the genus *Sylvilagus* where it causes only very minor cutaneous lesions. The first report of MYXV leaping host species from *Sylvilagus* to *Oryctolagus* rabbits was made in 1896 by Guisseppe Sanarelli but the identity of this apparently new veterinary pathogen as a novel poxvirus was not made until several decades later by the Brazilian microbiologist Henrique de Beaurepaire Aragao. Although MYXV is pathogenic only in European rabbits, the virus can replicate in cultured mammalian cells derived from a variety other species, including humans. The checkpoint for determining whether a poxvirus infection will be permissive or nonpermissive is usually made downstream from the binding/fusion/entry events, unlike many other viruses that depend upon specific host cell receptors for entry. It is believed that MYXV can effectively subvert all of the elements of the innate immune system of rabbits but cannot block all of these responses from non-lagomorphs, such as mice and humans. Like all poxviruses, MYXV expresses a wide array of immunomodulatory proteins, but only some of these are actually rabbit-specific when tested *in vitro*. At least one such species-nonspecific immunomodulatory protein derived from MYXV, a secreted virus-encoded serpin called SERP-1, has now completed human phase II clinical trials as an anti-inflammatory drug to treat acute coronary disease. Our studies on MYXV tropism also revealed that the virus is also permissive for a wide spectrum of human cancer cells. We are investigating the use of MYXV as an oncolytic virus to treat human cancers that exhibit defective signaling responses. In primary human cells, MYXV is highly sensitive to inhibition by several anti-viral cytokines, particularly type I interferon and tumor necrosis factor, whereas these anti-viral signaling pathways are frequently defective or absent in human cancer cells. We have recently shown that MYXV is very effective at eliminating disseminated human pancreatic cancer cells from xenografted immunodeficient mice. Also, we have begun to explore using MYXV to selectively infect and kill human cancer cells that contaminate bone marrow samples from patients with leukemia, myeloma or lymphoma but spare the normal $CD34^+$ hematopoetic stem and progenitor cells within the sample needed to reconstitute the immune system following autologous bone marrow transplantation. Thus, the fundamental study of a rabbit-specific poxvirus pathogen has revealed unexpected applications for treating human diseases as diverse as cardiovascular disease and cancer.

O005

EHEC O104:H4: phylogenetic origin and pathogenesis

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In the past early summer, we were confronted with the largest outbreak of hemolytic uremic syndrome (HUS) ever caused by an exceptionally virulent Shiga toxin (Stx)-producing *Escherichia coli* O104:H4 affecting more

than 850 patients and causing 53 death. In the past decades, this serotype has rarely been associated with HUS. The presentation will focus on the unique characteristics of this strain by comparing the outbreak isolates with other O104:H4 strains on the genome level and will discuss the pathogenetic traits of the EHEC O104:H4 outbreak strain. Interestingly, the current outbreak strain and the historical isolates associated with HUS carried genes typically found in two types of pathogenic *E. coli*, enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC). Phylogenetic analyses of the core genome genes indicated that the HUS-causing O104:H4 strains and the previously published sequence of the EAEC strain 55989 showed a close relationship but were only distantly related to common EHEC serotypes like O157:H7 or O26:H11. Though closely related, the outbreak strain differed from two historical strains isolated in the late 1990s and in 2001 in plasmid content and fimbrial genes that are related to virulence. Overall, the genomic data enabled an evolutionary model in which EAEC strain 55989 and EHEC O104:H4 strains evolved from a common EHEC O104:H4 progenitor, and suggest that by gain and loss of chromosomal and plasmid-encoded virulence factors and by additional point mutations, a highly pathogenic hybrid of EAEC and EHEC emerged.

O006

From STEC to HUSEC - EHEC yesterday and today

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Escherichia coli (*E. coli*) are well-known inhabitants of our intestinal flora of more than 200 described serotypes. Besides strains belonging to human commensal flora, there are different pathovars, comprising diarreagenic *E. coli* that are a worldwide important cause of Diarrhea. One important diarrheagenic group has in common the production of Shiga toxin and is therefore called STEC. The detection of STEC from humans, animals and environment is rising Europe-wide and especially in the Netherlands. In 2010, 400 STEC from human patients were reported in the Netherlands. STEC are higly diverse and can cause watery and bloody diarrhea as well as haemorrhagic colitis. In up to 30% of the cases of infection a severe extraintestinal complication, the Hemolytic Uremic Syndrome (HUS) can occur. The STEC associated with severe disease are also denominated Enterohaemorrhagic *E. coli* (EHEC). Conventional and molecular methods used in today's microbiological laboratories are not sufficient to distinguish an STEC from an EHEC infection. EHEC are generally distinguished from other STEC via serotyping, as they belong to certain serogroups (O157:H7/HNM, O26, O103, O111, O145, O91). Determination methods as Sorbitol MacConkey agar only detect the classical O157:H7 and fail in detecting the highly virulent O157:HNM and the other non-O157 EHEC serotypes. Anyway, the HUS-outbreak in Germany was caused by an *E. coli* O104:H4 and demonstrates that *E. coli* from other serotypes can cause HUS. Although, modern detection methods rely on the detection of Shiga toxin or genes coding for Shiga toxins in combination with an Intimin-coding gene (e.g. eae), the determination markers for the identification of *E. coli* associated with HUS (HUSEC) are necessary.

It has been shown that the severity of disease is associated with subtypes and amount of the Shiga toxin(s) produced by the strains. Only few Shiga toxin-variants seem to be clearly associated with HUS. In fact, the German outbreak strain harbored the classical Shiga toxin 2. His attachment properties seem to derive from other virulence and adhesion factors on the basis of his genomic background as Enteroaggregative *E. coli* (EAEC). In STEC detection and with STEC associated risk communication, STEC causing watery and bloody diarrhea need to be differentiated from EHEC potentially causing outbreaks of HUS. Patient treatment, infection control practice and public health action differentiates clearly between these two groups.

Although, Shiga toxin detection and subtyping helps in giving a risk assessment, more research on HUS-association needs to be done to clearly determinate HUSEC. New and innovative techniques need to be developed in order to improve the diagnostics and the management of the patient and a risk assessment. This is even more important, since stx-genes are also harbored by free bacteriophages and sometimes by other Enterobacteriaceae. Therefore, the characterization of a Dutch and European HUSEC reference-collection is essential for diagnostics, food safety and public health action. This will only be achievable by interdisciplinary research networks comprising efforts from human and veterinary medicine as well as from public health.

O007

Subtractive epidemiology of ESBL producing *Enterobacteriaceae* in the northern Dutch-German border region

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Introduction: Resistance in gram-negative bacilli is on the rise and treatment options are becoming scarce. However, little is known about the molecular epidemiology and transmission dynamics of these pathogens in different regions. In this cross-border EUREGIO-study, we compare the antibiotic resistance and molecular epidemiology of ESBL-producing *Enterobacteriaceae* isolates which were derived from Dutch and German hospitalized patients. We

aim to describe their population structure and antibiotic resistance patterns.

Methods: Within the prevention network EurSafety Health-net we prospectively examined 178 non-repetitive isolates, obtained between January and July 2011 from inpatients in four tertiary-care hospitals representing the four largest hospitals in the Northern Dutch-German border region: Groningen (n = 68), Enschede (n = 20), Münster (n = 34) and Oldenburg (n = 56). All samples were phenotypically analysed for ESBL using Vitek 2 and the antibiotic resistance was determined with MIC values according to EUCAST. Resistance was confirmed by PCR on target genes. Diversilab (DL)-system, a semi-automated repetitive-sequence-based PCR typing system for rapid genotyping, was used for all ESBL positive samples.

Results: PCR confirmed 123 *E. coli*- and 55 *Klebsiella pneumoniae* producing ESBL species. Antibiotic resistance showed significantly more resistance in the German compared to the Dutch isolates for: Piperacillin+Tazobactam (75% vs 18%, Fisher's exact test; $p < 0.0001$ respectively) and Ciprofloxacin (71% vs 51%, $p = 0.033$ respectively). There was no difference for carbapenems or aminoglycosides.(see Table 1)

Molecular typing revealed 25 different clusters for *E. coli* comprising 75% of all isolates and 34 sporadic *E. coli* isolates. All clusters were distributed equally in all four hospitals and regions. There was one big cluster containing 33 isolates of all studied regions.

In contrast, typing of *Klebsiella pneumoniae* isolates showed 17 clusters comprising 76% of isolates which were mainly regionally disseminated. There were 27 sporadic isolates comprising 34% of all isolates.

Conclusion: This comparative cross-border study revealed that German ESBL strains are more resistant to antibiotics than Dutch strains. The differences of clonal distribution of *E. coli* and *Klebsiella pneumoniae* suggest that *Klebsiella pneumoniae* is spread nosocomially while *E. coli* is spread via different routes independent of hospital stay or country. Further studies need to be done.

O008

High acquisition rates of ESBL producing *Enterobacteriaceae* among Dutch travelers

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Background: The increasing rate of resistance in *Enterobacteriaceae* is a major cause of concern. Unprecedented human air travel and migration allow multiresistant clones and plasmids to be transported rapidly between countries and continents. Foreign travel has been demonstrated to be a risk factor for colonization with ESBL-producing *Enterobacteriaceae*.

Objective: To study the fecal acquisition of ESBL producers and risk factors among Dutch travelers.

Study design: From March 2011 to September 2011, a prospective follow-up study was conducted at the travel clinic of Leiden University Medical Center (LUMC) and Municipal Health Service Leiden, the Netherlands. Healthy Dutch volunteers travelling outside Europe were enrolled. Data on potential travel-associated risk factors and rectal swabs were collected before and after traveling. Rectal swabs were cultured on chromID ESBL agar after preculturing in selective broth. ESBL confirmation was performed with combination disk synergy testing of ceftazidim with clavulanic acid. Isolates are currently analysed with a DNA microarray Check-MDR CT103 for the presence of ESBLs, plasmid mediated ampCs and carbapenemases.

Results: A total of 473 travelers were included. Thirty-three participants carried ESBLs before travel and were excluded from the analysis. The data of the first 307 Dutch travelers, who completed the study, are described here. A total of 107 participants with negative pre-travel samples were colonized with ESBL-producing *Escherichia coli* (n = 100), *Klebsiella pneumoniae* (n = 4) or both (n = 2), or *Enterobacter cloacae* (n = 1) after the trip. Despite the small number of travelers to India (n = 16), multivariate analysis showed that this was associated with the highest risk factor for the acquisition of ESBLs (OR 8.4). Travel to other destinations was associated with the following rates of posttravel ESBL colonization: 42% for Asia (India excluded), 26% for Africa, 25% for the Middle-East and 17% for Soutern/Middle America. Staying in budget hotels showed a positive trend for acquiring ESBLs. Gastroenteritis during the trip was not a significant risk factor.

Conclusion: This study found a very high fecal carriage of 35% ESBLs among Dutch travelers. The highest acquisition rate was found in travelers to India. In this study population, gastroenteritis during the trip was not associated with the acquisition of ESBLs. We found a higher pretravel fecal ESBL carriage of 11% than we had expected from earlier data.

O009

Emergence of West Nile virus in Greece

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West Nile virus (WNV) is a mosquito-borne flavivirus causing to humans an asymptomatic or mild disease (approx.80% and 20% of infections, respectively), while in less than 1% of infections there is involvement of the nervous system, affecting mainly the elderly persons with

an underlying disease. WNV is endemic in many regions in Africa, Europe, Asia and North America, and causes sporadic cases or outbreaks.
A large WNV outbreak occurred in summer of 2010 in Greece, starting near a river delta in northern part of the country. Previous studies had showed approx. 1% seroprevalence among humans, while no WNV case had been previously reported in Greece. In a serosurvey of 2007, WNV neutralizing antibodies had been detected in 4/392 residents of the region where was the focus of the outbreak in 2010. Nine prefectures in northern Greece were affected in 2010, with most cases being observed in Central Macedonia. Apart the numerous mild cases, 197 neurological (88% encephalitis) cases were laboratory diagnosed, 33 of them (17%) fatal. The median age of the patients with neuroinvasive disease was 72 years (12-88 years). The incidence of the neurological disease was 15 per 100,000 citizens. Many additional patients presented with a febrile disease, usually accompanied by exanthema. Molecular testing of mosquitoes collected at the sites where the cases were observed showed that the strain belonged to WNV lineage 2. Whole genome nucleotide sequencing showed that the genetically closest WNV strain was that detected in 2004 in birds in Hungary, differing from it by 44 nucleotides, with one of them resulting in mutation H249P in NS3 gene, which has been previously associated with increased pathogenicity in WNV lineage 1 strains. Identical sequences were recovered from blood donors, as well as in additional mosquito pools, in wild birds and, later, in spring 2011, in sentinel chickens. WNV outbreak occurred for a second consecutive year, in 2011. Apart the mild cases, 76 neuroinvasive cases have been reported, 8 of them fatal. This year the incidence was lower (0.68/100,000), the cases were more dispersed (North and Central Greece), and the fatality rate was lower (10.5%). Many studies on WNV followed, concerning the development time of the IgG antibodies, the persistence of the IgM antibodies, the cross-reactivity, the clinical course of the non-neuroinvasive cases, while a follow-up study is currently ongoing. The circulating WNV strain of 2011 was identical with that of 2010, and further studies will show the level of its pathogenicity.

O010

West Nile virus non-coding RNA: effects on pathogenicity and mosquito transmission

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Flaviviruses like West Nile, Japanese encephalitis and dengue are highly pathogenic RNA viruses that are transmitted to humans via mosquito bites. In humans and other vertebrates, flavivirus infections can cause encephalitis or hemorrhagic fevers, but in mosquitoes the infection process is non-pathogenic and persistent. While very high viral loads are found in mosquito salivary glands, infection has limited impact on mosquito lifespan and blood feeding activity, a likely result of natural selection to enhance virus transmission in the field. Flaviviruses have long been thought to have a relative simple genome, consisting of a single viral RNA encoding a single polyprotein. However, studies on West Nile virus have shown that an additional, non-coding viral RNA species strongly accumulates during virus replication. Detailed molecular studies demonstrated that this so-called subgenomic flavivirus RNA (sfRNA) of approximately 0.5 kilobases is abundantly produced in West Nile virus infected cells. Most importantly, sfRNA was shown to be essential for virus-induced cytopathicity and viral pathogenicity in a mouse model. Despite its clear importance in pathogenesis, the biogenesis and exact molecular function of sfRNA remain elusive. The observation that sfRNA is produced to very high levels in mosquito cells and the recent insight that sfRNA is a precursor molecule for a viral microRNA in cells of mosquito origin, both implicate a crucial role for sfRNA in mosquito infection. Our current research is therefore concentrated on how sfRNA modulates non-pathogenic flavivirus replication in Culex pipiens mosquitoes and how it contributes to West Nile virus transmission in general. Ultimately, this research may aid the generation of antiviral compounds interfering with sfRNA production and the rational design of live-attenuated flavivirus vaccine candidates.

O011

The role of immature virus particles in dengue pathogenesis

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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 and Dengue shock syndrome. 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 antibodies. These antibodies facilitate efficient binding and cell entry of immature particles into

Fc-receptor expressing cells. In addition, enzymatic activity of furin present in the endosome is critical to render the internalized immature virus infectious. In this study, we analyzed if antibodies recognizing the E protein can also promote viral infectivity of immature virus particles.
Methods: Immature DENV-2 strain 16681 particles were produced in furin-deficient LoVo cells. The infectious properties of immature and standard DENV-immune complexes were investigated in FcR-expressing human monocyte cell line U937, the murine macrophage cell line P388D1, and in human PBMCs by plaque assay in the presence and absence of furin inhibitor.
Results: The vast majority of anti-E mAbs tested enhanced viral infectivity of immature dengue in a furin-dependent manner. Furthermore, we found that in the presence of non-neutralizing immune serum, immature virions, which are normally non-virulent, can cause lethal disease in mice.
Conclusions: Most of the E antibodies tested facilitated binding and uptake of immature virions into an endocytic pathway of the target cell. While most antibodies promoted infection, some did not. Anti-E mAbs that do not stimulate viral infectivity may interfere with the conformational change of the virion prior to furin cleavage, or with the fusion process itself. Taken together, our results support the notion that antibodies against the structural proteins prM and E both can enhance infectivity of prM-containing immature and partially mature flavivirus particles. We are currently running experiments using acute sera samples of patients developing different disease outcomes to assess whether immature particles contribute to disease pathogenesis and we anticipate to present these results at the conference as well.

O012

Detection of Dengue NS1 in travelers: a comparison of the performance of three rapid diagnostic tests with the Platelia NS1 antigen ELISA

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Introduction: Dengue virus non-structural protein 1 (NS1) has been identified as an early marker of acute dengue infection. The Platelia NS1 ELISA (Platelia NS1) from BioRad is generally regarded as the best assay available to test for NS1. There are also three commercial rapid diagnostic tests that detect NS1 antigen (Ag) in serum and that can be used for point-of-care testing (POCT). The performances of these tests have not been compared with the Platelia NS1 in Dutch travelers suspected of dengue.
Methods: To evaluate the performances of Dengue NS1 Ag strip (Bio-Rad Laboratories), Dengue DX NS1 Ag (Focus Diagnostics, Standard Diagnostics), and Dengue Early Rapid (Panbio) in comparison with Platelia NS1 we included 272 stored paired serum samples from 136 travelers with febrile illness upon return to the Netherlands between 2000 and 2011. 94 patients had acute dengue based on NS1 positivity in the Platelia NS1 in the primary sample and seroconversion or IgG titer elevation in the convalescent sample; 14 patients had a recent dengue infection based on either the presence of IgM in the primary sample or an increase in IgG titer in the convalescent sample. The primary samples of these 14 patients, however, tested negative for NS1; 28 patients were IgM positive in the primary sample but showed no seroconversion in the convalescent sample. These patients were regarded as not having had an infection with dengue, but with a related virus. We tested all 136 primary samples for NS1 with the above tests. To obtain an impression of the analytical sensitivity of the NS1 assays, we performed a serial dilution end point titration for all four dengue serotypes.
Results: The Dengue NS1 Ag strip detected 75 of 94 Platelia NS1 positive samples and there were no false positive tests. The Dengue DX NS1 Ag detected 72 of 94 Platelia NS1 positive samples; one test was false positive. The Dengue Early Rapid test detected 72 of 94 Platelia NS1 positive samples; two tests were false positive. In our panel, the sensitivity and specificity of Platelia NS1, Dengue NS1 Ag strip, Dengue DX NS1 Ag, Dengue Early Rapid were respectively 87%, 69%, 67%, 67% and 100%, 100%, 96%, 93%. The positive and negative predictive values were respectively 100%, 100%, 99%, 97% and 67%, 46%, 43% en 42%. The ability to detect NS1 in diluted serum samples varied greatly for each assay and between serotypes. Only the Platelia NS1 was capable of detecting NS1 from all serotypes in 1/200 dilution.
Conclusions: This study demonstrates that rapid diagnostic tests detecting NS1 are less sensitive than the Platelia NS1. The performance of the three rapid diagnostic tests is not markedly different, but in our panel the Dengue NS1 Ag strip from Bio-Rad Laboratories had the highest sensitivity and specificity.

O013

An outbreak of histoplasmosis misdiagnosed as miliary tuberculosis among participants of a tropical biology course in Uganda

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Introduction: We describe two cases of histoplasmosis in travellers to Uganda who were misdiagnosed as miliairy tuberculosis. They were part of an international group of students who attended a field course on tropical ecology in Kibale Forest National Park.

Case 1. A previously healthy 25-year-old man presented with fatigue, cough, dyspnea en fever. The C-reactive protein (CRP) was 221 mg/L, white blood cell count (WBC) was 8.8 /nl. One week earlier he returned from a 1 and a half month stay in Uganda. Complaints started in Uganda and he was hospitalized there for a few days. Chest X-ray and high resolution computed tomography showed bilateral small nodular infiltrates resembling miliary tuberculosis. Tuberculosis treatment was started and diagnostic tests for tuberculosis were ordered. Urine samples, bronchial lavage fluid, bone marrow biopsy and liver biopsy were examined by auramine staining, polymerase chain reaction (PCR) and culture but were all negative. Histopathological examination of the liver biopsy showed granulomas without acid-fast bacteria. The Interferon-gamma release assays (TBC-IGRA) was negative. After initial worsening the patient recovered. Meanwhile he received e-mails that other course participants where also hospitalized all over the world with similar symptoms, however, no diagnosis was made in these cases as well. At that time we suspected histoplasmosis and indeed the Histoplasma Antigen in urine was positive and the Histoplasma serology performed by the immunodiffusion test was weakly positive. Histoplasma capsulatum PCR on serum was negative. Histoplasma was not cultured from the bronchial lavage fluid, bone marrow or liver biopsy. The patient was treated with itraconazol and recovered fully.

Case 2. Two months after patient 1 we saw another course participant, a 23-year-old previously healthy woman. After the course she continued travelling through Africa and fell ill almost at the same time as patient 1. She experienced fever, cough, myalgia and weightloss. Three weeks later in Kenya she heard that patient 1 was hospitalized with presumably tuberculosis. She went to see a local physician and after a CT scan showed multiple small nodular lesions in the lung was started on treatment for tuberculosis. After a few days she felt somewhat better and continued her journey. When we examined her she was fully recovered. WBC was 6.1/nl, CRP was 18 mg/L. Tuberculin skin testing and TBC-IGRA were negative. Histoplasma immunodiffusion test was weakly positive, Histoplasma Antigen in urine was negative. Tuberculosis treatment was stopped and since she had fully recovered no Histoplasmosis treatment for was started. The students who became ill were probably exposed to a high fungal burden while exploring a hollow tree inhabited by many bats.

Conclusion: We describe a histoplasmosis outbreak among students participating a field course in Uganda. Radiographic imaging of pulmonary histoplasmosis resemble those of miliary tuberculosis. Histoplasmosis should be considered in healthy travellers returning with fever from endemic areas like (sub)tropical areas or the USA.

O014

Outbreak of a multi-drug resistant *Pseudomonas aeruginosa* on the intensive care unit of a tertiary care hospital in the Netherlands; a case-control study to identify sources and risk factors

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Objectives: In October 2010 four patients on the intensive care unit (ICU) had positive cultures with a *Pseudomonas aeruginosa* strain that was resistant to ceftazidime, gentamicin, tobramycin and meropenem, but susceptible to colistin. Retrospectively, another 13 case patients could be identified from 2009 onwards, of which 11 had been admitted to the ICU. Despite intensification of hygiene measures, the epidemic continued until November 2011, with 44 cases in total. Typing by AFLP showed the *P. aeruginosa* strains of these patients to be clonal in two clusters. The larger cluster (n = 30) encompassed strains that were ciprofloxacin susceptible, the isolates of the smaller cluster (n = 12) were ciprofloxacin resistant (n = 2 patients had both strains). Tested isolates produced a VIM-carbapenemase. To identify a common source and factors associated with acquisition, an extensive case-control study was conducted.

Methods: Patients who were colonised or infected with the *P. aeruginosa* outbreak strain between January 2010 and August 2011 - irrespective of the cluster – were included in the study (n = 35). Patients with no prior history of ICU admission and patients with a positive culture within 48 hours of admission were excluded (n = 3). Sixty-four control patients from the ICU were selected for these 32 cases. Control patients were matched for day of admission and length of stay. Case and control patients were compared with respect to individual and admission data, procedures during ICU stay, ventilation parameters and use of antibiotics.

Results: Matched univariate regression analysis revealed surgery, admission on subunit 1, central venous catheters and warming-up with a warm-air tube as risk factors. With multivariate conditional logistic regression analysis, surgery (OR 5.7 95% CI [1.6-20]), admission on subunit 1 (OR 6.1 95% CI [1.7-22]) and warming-up procedure (OR 3.6 95% CI [1.2-11]) were independently related to becoming a case patient. From this study and by descriptive epidemiology, two rooms on the ICU (89 and 113), two operation theatres, two ventilation machines and one hemofiltration machine were additionally identified to be potentially associated with case patients. Environmental sampling including these machines, as well as warm-air tubes on the ICU and Blanketrols® in the operation rooms, revealed that the faucet aerators in patient rooms 89 and 113 were positive with the *P. aeruginosa* outbreak strain.

Further enforcement of hygiene and isolation measures and renewal of the faucets have controlled the epidemic so far.

Conclusion: Faucets on the ICU may have served as a source for the transmission of a multi-drug resistant *P. aeruginosa*. Patients who underwent surgery and were warmed-up were at highest risk. Cross-transmission between healthcare workers and patients is the most likely way of further spread, as stringent hygiene measures have limited the epidemic so far.

O015

The curious case of a man who slept in the daytime

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We describe the case of a 38-year old male patient who presented with unexplained progressive somnolence, forgetfulness and bradyphrenia, and the ultimate elucidation of its microbiological cause.

The symptoms were first noticeable 6 month prior to presentation, but had recently increased to include hallucinations, a nocturnal tremor and pain in both legs. There was no dysfunction with regards to the senses or muscle strength and he had no headache. Appetite, intake and digestion were normal and the patient's weight remained stable. Other than bradyphrenia and dysarthria, no other gross abnormalities were evident upon physical or neurological examination.

Haematological and blood biochemistry parameters were unremarkable, with only marginally elevated inflammatory markers. Investigation of cerebrospinal fluid showed a mild non-specific leukocytosis. Cerebral radiological examination revealed multiple small (< 1cm) mass lesions of the suprasellar hypothalamus, with rim-enhancement and peripheral oedema. Other than enlarged mediastinal and mesenteric lymph nodes and splenomegaly, no other abnormalities were observed.

Histopathological examination of lymph nodes and a crista biopsy provided no additional diagnostic clues. Blood cultures were repeatedly negative, as was serology for amongst others HIV, toxoplasma, syphilis, tuberculosis and cystercercosis; a PCR for *Trophyrema whipplei* on whole blood was also negative. Similarly, cerebrospinal fluid was negative by PCR for herpesviridae, enteroviridae, TBC, *T. whipplei* and using pan-microbial 16S RNA primers.

Following deterioration of his neurological condition and the radiological findings, the patient was treated empirically with intravenous cefotaxim and metronidazol, upon which some clinical improvement was observed. However, the finding of PAS-positive, Grocott-negative, although gram-negative, micro-organisms upon histopathological examination of a brain biopsy, led to the reconsideration of Whipple's disease as a possible cause. Although both 16S RNA pan-bacterial and 18S RNA pan-fungal PCRs on biopsy material were negative, a specific *T. whipplei* PCR finally confirmed the diagnosis.

First described as a rare post-mortem finding in 1907, Whipple's disease usually affects the small intestine, causing malabsorption and eventually, if left untreated, death. The causative organism, a gram-positive rod related to the Actinomycetes, was only identified in the 1990's. Risk factors include male sex, middle age, Caucasian race and agricultural activities. Although extra-intestinal manifestations, including endocarditis, pathognomonic occulomasticatory myorrhythmia and encephalitis are relatively common in conjunction with gastrointestinal disease, their isolated occurrence is extremely rare. A recently published case series, however, reported excellent clinical improvement of neurological manifestations upon treatment with doxycycline and hydroxychoroquine plus a sulfa drug, but recommends life-long doxycycline prophylaxis to prevent (frequent) recurrences.

We conclude by discussing the key diagnostic clues in this case -in particular the histopathological findings- and setting out recommendations for early recognition and treatment of future cases of cerebral Whipple's disease in the Netherlands.

O016

Appendiceal spirochaetosis in children

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Introduction: Appendicitis is a well-known surgical emergency, diagnosed most often in children. Despite thorough pre-operative diagnostic protocols, between 17 and 37% of removed appendices do not show any histopathological signs of inflammation. It has been hypothesized that infections with spirochaetes might explain the clinical symptoms in these not-acutely inflamed appendices. However, reports mentioning appendiceal spirochaetosis have been predominantly focussed on adults, with varying incidences (0.3% - 12.3%). While acute appendicitis occurs most frequently in children, no reliable data exists for this group.

Aim: To investigate the incidence of Brachyspira species in paediatric appendicitis by histopathology and realtime PCR.

Materials and Methods: Formalin-fixed paraffin-embedded appendix resection specimens of paediatric patients (< 18 years), obtained from 2 Dutch Hospitals between 1988 and 2011 were selected. Three clinicopathological groups were identified: clinical acute appendicitis, histopathologically confirmed (n = 61), clinical acute appendicitis without

inflammatory changes (n = 57) and appendices removed per occasionem (n = 34). Groups were matched regarding age and gender. Appendices were processed using a semi-automatic protocol supplied by Siemens Healthcare. PCR reactions were performed using a Brachyspira-specific real-time PCR on a Roche LightCycler 480 II. Two internal controls were used for quality of DNA (β-globulin) and DNA extraction (Phocine Herpes Virus). All samples were microscopically verified by routine H&E staining and confirmed with immunohistochemistry (*Borrelia*-staining).
Results: No statistical differences existed between the included groups regarding age, sex and histopathological conclusion. Eight samples had to be excluded because of insufficient quality of DNA. Five samples were positive for Brachyspira: 1 patient with a histopathological confirmed acute appendicitis (6-12 years), 2 in the histopathological no-inflammation group (12-18 years) and 2 in the per occasionem group (12-18 years). Sequencing indicated 3 Brachyspira aalborgi and 2 novel Brachyspira species. Brachyspira were found in 5 of 76 male but never in the 67 female patients (p = 0.04).
Conclusions: To our knowledge, this is the first study investigating appendicael spirochaetosis in children. Brachyspira species are not increased in appendices of children with clinical signs of acute appendicitis. This indicates that Brachyspira species do not play a causative role in the pathogenesis of acute appendicitis in children.

O017

Asymptomatic carriage of *Mycoplasma pneumoniae* in the upper respiratory tract of children

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Introduction: Although *Mycoplasma pneumoniae* is regarded as a common cause of respiratory tract infections (RTIs) in children, little is known about the prevalence of asymptomatic *M. pneumoniae* carriage. Our primary objective was therefore to determine the prevalence of *M. pneumoniae* in children in the absence of RTI symptoms.
Methods: 412 asymptomatic children and 321 children with RTI symptoms were enrolled between July 2008 and November 2011. Extensive sampling was performed on all children, which included pharyngeal and nasopharyngeal samples and capillary blood samples. This allowed us to detect serum-antibodies against *M. pneumoniae* by ELISA, to detect and quantify genomic copies of *M. pneumoniae* by real-time PCR, and to detect other bacterial and viral respiratory pathogens.
Results: *M. pneumoniae* DNA was detected in 20.7% of the asymptomatic children and in 16.7% of the children with an RTI. There was no significant difference between the two groups in distribution of genomic copy number or in prevalence of anti-*M. pneumoniae* serum-antibodies. Interestingly, the presence of serum-antibodies did not correspond to real-time PCR results. Longitudinal follow-up showed the persistence of *M. pneumoniae* in the absence of symptoms for up to 4 months. Other pathogens were also found to be commonly present in the respiratory tract of asymptomatic children
Conclusion: The presence of *M. pneumoniae* DNA in the upper respiratory tract is not indicative for symptomatic RTI in children. In addition, the mere detection of pathogens in the upper respiratory tract is generally not sufficient to determine the aetiology of an RTI.

O018

Pneumococcal pneumonia: the association between clinical severity and serotype

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Introduction: *Streptococcus pneumoniae* is the most common cause of community acquired pneumonia worldwide. Forty percent of the pneumococcal pneumonia cases are accompanied by a bacteremia as detected by blood culture. The clinical severity of a bacteremic pneumococcal pneumonia is not uniquely precarious. It can vary from mild respiratory disease to septic shock or even death. This spectrum of clinical severity is the resultant of pathogen characteristics and the host's response during infection. In the bloodstream, pneumococci are mantled by a polysaccharide capsule that facilitates immune evasion. In this study, we investigated the association between the structural composition of this pneumococcal polysaccharide capsule (serotype) and the clinical severity of a bacteremic pneumococcal pneumonia.
Methods: Retrospectively, we included 124 patients who were hospitalized with a bacteremic pneumococcal pneumonia between 2001 and 2008. The serotypes of the pneumococci that were isolated from blood cultures were determined by capsule locus PCR analysis. The clinical severity of disease was measured by the Pneumonia Severity Index (PSI) at admission, the development of sepsis, the admission to Intensive Care Unit (ICU) and in hospital mortality. The mean PSI score per serotype was compared with the PSI score of the total population.
Results: Within the total patient cohort, the mean PSI score at admission was 108.8, which correlates with risk class 3 out of 5. Twenty-two percent of the patients developed sepsis (n = 27), 19% (n = 23) was admitted to the ICU and in hospital

mortality was 12% (n = 15). The 124 pneumococci represented 22 different serotypes. Pneumococci of serotype 8 caused bacteremic pneumonia with a significantly lower PSI score compared with the total cohort (88.9 vs. 108.8, risk class 2 vs. 3, p = 0.0082). Bacteremic pneumonia caused by serogroup 6 had a significantly higher mortality rate compared with the total cohort (42% vs. 12%, p = 0.017). No associations were found between pneumococcal serotype and the development of sepsis or ICU admission.

Conclusion: Our results indicate that bacteremic pneumonia caused by pneumococcal serotype 8 is associated with relatively mild disease, whereas those caused by serogroup 6 are associated with a relatively high mortality rate. Currently, the study cohort is expanded to 266 bacteremic pneumococcal pneumonia patients. Importantly, besides the capsular contribution to disease severity, the importance of additional pneumococcal genes will be investigated by comparative whole genome sequencing.

O020

WIP guidelines on multiresistant microorganisms in hospitals

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The WIP (werkgroep infectiepreventie) develops national guidelines on infection prevention in hospitals. Recommendations for isolation forms, disinfection procedures, contact investigation and the institution of an outbreakmanagement team for multiresistant micro-organisms are described in two separate WIP-guidelines: BRMO (in Dutch: 'bijzonder resistente microorganismen') and MRSA (in Dutch: 'Meticillineresistente *Staphylococcus aureus*').

Recently, the WIP revised these two guidelines. Both draft guidelines are at this moment open for comments by infection prevention professionals. These professionals are responsible for the implementation of these guidelines in the hospitals. The major changes in the BRMO guideline will be discussed during the presentation.

In case of an outbreak the WIP guidelines provide specific infection prevention measures for BRMO and MRSA in combination with the WIP guidelines on standard infection prevention procedures. The installment of two permanent WIP expert groups, one on BRMO and one on MRSA, make it possible to assure a quick and adequate response to outbreak situations and changes in the field of infection prevention on multiresistant microorganisms. As an example, actions taken by the WIP in response to the Maasstad hospital outbreak will be presented.

O021

Responsibilities in an outbreak situation: is appropriate action hampered by legal rules or restrictions?

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When faced with a longer ongoing outbreak of an anti-microbial resistant pathogen, recent incidents show that response policies cannot be confined to the originating hospital. The ever faster forwarding of patients make that nursing homes, other hospitals and their outpatients are directly involved. In addition to this, public and political concern is expressed and public health authorities become involved, as experts in outbreak control situations. Co-ordinated action is prerequisite and demands as a consequence the exchange of personal and medical data of patients.

Lately, questions are raised about the legality of sharing these personal data of patients. Some professionals underline their medical confidentiality and refuse to share data when not solely in the direct interest of the individual patient; others make a distinction between medical colleagues and public health authorities; again others underscore the public's interest of sharing all necessary data without restraint.

This presentation analyses the respective responsibilities of parties and the applicable rules of medical, administrative, and public health law. The scarce jurisprudence will be presented and collision of ruling will be clarified. Possible legal gaps are identified and discussed, to contribute to future consensus on an appropriate legal framework for co-ordinated action.

O023

National surveillance of outbreaks, are you crazy?

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The Netherlands has a long tradition in well-organized and effective infection control. Hospital-acquired MRSA is the typical example of this success, which is close to non-existent in the Netherlands. So why bother to introduce a national system for surveillance of hospital outbreaks?

Recently the emergence of multi-resistant gram-negative rods has shown that detection, confirmation and control of these BRMO is not always straightforward. In addition, via ISIS-AR it was shown that spread of certain BRMO could occur in one or more hospitals, without being noticed by microbiologist, infection control nurse or physician.

For the control of BRMO, physicians and other healthcare workers have to comply with guidelines of the infection control unit. Because of the long experience with MRSA

and the legal backup by a national guideline, this is not a big issue for MRSA. However, for new BRMO the compliance may not always be as easy to enforce.
Because of the impact an undetected or uncontrolled outbreak of resistant microorganisms could have for hospital and community a new structure was designed with the intention to provide information and if necessary help for hospital outbreaks with resistant microorganisms. Each month a panel of experts will discuss all available information regarding outbreaks. If necessary, the infection control unit will be contacted to provide extra information and to offer help if needed. The intention is to organize information and support by professionals, which will help to improve the quality of infection control in the Netherlands. This structure is based on sharing of information and therefore confidentiality is crucial. We will describe how we envision this structure might be organized.

O024

Bacterial sensors of oxygen: lessons taught by *Escherichia coli*

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Oxygen availability has profound effects on bacterial central metabolism. Many enteric pathogens are facultative anaerobes and switch between three basic metabolic modes (aerobic respiration, anaerobic respiration and fermentation) depending on the presence or absence of different electron donors and electron acceptors. Furthermore, the expression and or activation of some virulence factors can be regulated by oxygen supply. Such changes in physiology are ultimately rooted in altered patterns of gene expression. The Fumarate and Nitrate Reduction regulator (FNR) is an oxygen-responsive transcription factor that is conserved amongst many enteric pathogens (Korner *et al*. 2003). FNR has been shown to be a global regulator of virulence and anaerobic metabolism in *Salmonella enterica* serovar Typhimurium (Fink *et al*., 2007) and oxygen-regulated FNR activity is necessary for the synthesis and subsequent function of a virulence critical type three secretion system (TTSS) in *Shigella flexneri* (Marteyn *et al*., 2010) and the cytolysin HlyE (Hunt *et al*., 2009). Thus, the mechanisms of oxygen-sensing and transcription regulation by FNR are of interest.
In the absence of oxygen FNR forms a DNA-binding, transcriptionally active, dimer in which each protomer possesses an oxygen-sensitive [4Fe-4S] cluster. When oxygen is present the iron-sulphur clusters are degraded to form [2Fe-2S] clusters via a [3Fe-4S] intermediate with concomitant conformational changes that drive transfer to a non-DNA-binding transcriptionally inactive state (Crack *et al*. 2007; Jervis *et al*., 2009). The FNR [4Fe-4S] cluster is exquisitely sensitive to oxygen and this sensitivity can be tuned by protein environment surrounding the iron-sulphur cluster. In its active, anaerobic, form FNR recruits RNA polymerase to target promoters to activate transcription. The range of genes regulated is determined by the availability of oxygen and transcript and metabolite profiles for *E. coli* indicate that there is a progressive switch from aerobic respiration to anaerobic fermentation as the oxygen supply is lowered (Rolfe *et al*., 2011).
The *Shigella* TTSS regulation is a striking demonstration of the importance dynamics. A systems biology approach to investigate adaptation of *E. coli* cultures during transitions between aerobic and anaerobic environments has revealed that the oxygen-responsive regulatory network extends beyond the direct influence of FNR to include transcription factors such as PdhR that react to changes in metabolite concentrations that arise as a consequence of the perturbations in oxygen availability (Trotter *et al*., 2011).
These, and other, issues related to oxygen-perception and the consequences for bacterial behaviour will be discussed.

References

- Crack, J.C. *et al.* (2007) Proc Natl Acad Sci USA. 104, 2092-2097
- Fink, R.C. *et al.* (2007) J Bacteriol. 189, 2262-2273
- Hunt, S. (2010) Adv Exp Med Biol. 677, 116-126
- Jervis, A.J. *et al.* (2009) Proc Natl Acad Sci USA. 106, 4659-4664
- Korner, H. *et al.* (2003) FEMS Microbiol Rev. 27, 559-592
- Marteyn, B. *et al.* (2010) Nature. 465, 355-358
- Rolfe, M.D. *et al.* (2011) J Biol Chem. 286, 10147-10154
- Trotter E.W. *et al.* (2011) PLoS ONE. 6, e25501

O025

General and oxidative stress responses in foodborne pathogens

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Bacteria have evolved various strategies and networks to survive and adapt to changing conditions including those encountered during processing of foods and during infection of the host in case of pathogenic bacteria. Survival capacity of foodborne pathogens allows them to withstand a wide range of stresses including low pH, disinfectants and antibiotics. Most of the notorious human pathogens prefer an aerobic lifestyle that is intrinsically associated with the activation of oxidative defense mechanisms since low levels of reactive oxygen species (ROS) are inevitably produced as a byproduct of electron transfer chain activity and cellular metabolism. Disturbance of cellular homeostasis leading

to inhibition of growth and/or inactivation of pathogenic bacteria is a unifying concept in both food preservation and medical microbiology and knowledge on bacterial behavior may supply tools for enhanced control. Integration of information contained in comparative transcriptome analysis, wild type versus deletion mutant performance, promoter reporter studies, single cell analysis using fluorescence microscopy and flow cytometry, has provided a basis for understanding bacterial adaptive responses and cross protection provided by a range of stresses relevant in a variety of settings. Based on our studies with *Listeria monocytogenes* and toxin producing *Bacillus cereus*, two issues will be highlighted including the impact of primary oxidative stress on activation of SOS response-mediated DNA damage repair and generation of antibiotic resistant variants, and the role of so-called secondary oxidative stress with concomitant generation of ROS causing bacterial cell death.

References

- Mols M, Abee T (2011) Primary and secondary oxidative stress in Bacillus. Env. Microbial. 13(6):1387-1394
- van der VeenS, Abee T (2011) Bacterial SOS response: a food safety perspective. Curr. Opinion Biotechnol. 22(2):136-142
- Abee T, Wels M, de Been M, den Besten H (2011) From transcriptional landscapes to the identification of biomarkers for robustness. Microbial Cell Factories Aug 30;10 Suppl 1:S9

O026

Translational regulation of the respiratory electron transport chain of *Neisseria meningitidis* by the Fur controlled small non-coding RNA NrrF

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So far NrrF is one of the three identified small non coding RNAs from the strictly human pathogen *Neisseria meningitidis* and was previously shown to mediate post-transcriptional repression of succinate dehydrogenase (*sdhCDAB*) under control of the ferric uptake regulator (Fur). To determine the full extent of NrrF mediated regulation in *N. meningitidis* we combined a biocomputional target mRNA prediction with experimental approaches. In silico, mRNA of *petABC* and *cycA*, encoding for the cytochrome bc1 complex and cytochrome c4 respectively and both functionally involved in respiration, were identified as putative targets of NrrF. Using heme-stained protein blots we show that expression of both proteins decreased in meningococci overexpressing NrrF. In addition, NrrF mediated down regulation of expression of CcoO, CcoP, cytochrome c5 (cycB), also components of the respiration chain, and CycP was also observed. Direct interaction between NrrF and the 5'- untranslated region of the mRNAs of *petABC, cycA, cycB, cycP,* and *ccoNOQP* was assessed *in vivo* by using a *gfp* reporter system for translational control and target recognition of sRNAs in *E. coli* and so far confirmed for *petABC*. Together, our results provide for the first time insight into the mechanism by which components of the respiratory chain are regulated by Fur. Adaptation of expression of these components to iron limitation is mediated at the post-transcriptional level through the action of the small regulatory RNA NrrF.

O027

Genome-wide identification of *Streptococcus pneumoniae* genes essential for growth and survival in CO_2-poor environmental conditions

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Introduction: The spread of *S. pneumoniae* to new human hosts is a prerequisite for the persistence of this respiratory tract pathogen in the community. Pneumococcal transmission has also contributed to the significant rise of antibiotic resistance and vaccine escape observed over the recent decades. Hence, knowledge on pneumococcal transmission factors could contribute to the development of novel strategies to treat and prevent pneumococcal disease.
Methods: To identify pneumococcal transmission factors, we exposed *S. pneumoniae* mutant libraries to conditions reflecting the environment encountered by the pathogen outside the host. Mutants that failed to survive under these conditions were identified by the Tn-seq next generation sequencing technology, and signify genes essential for *S. pneumoniae* transmission.
Results: Tn-seq analysis of a 40,000 CFU *S. pneumoniae* R6 *mariner* transposon mutant library exposed to CO_2-poor (0.035%) environmental and CO_2-rich (5%) host conditions reproducibly identified 10 mutants specifically attenuated for pneumococcal growth in the CO_2-poor environment. Only 2 genes, encoding the pneumococcal carbonic anhydrase (PCA; spr0026) and a dihydrofolate synthase (FolC; spr0178), were represented by multiple unique mutants. Validation experiments with *S. pneumoniae* directed mutants confirmed the essential role of the *pca* and *folC* genes for growth in CO_2-poor environmental conditions. Pneumococcal carbonic anhydrases appeared to mediated the reversible hydration of CO_2 to HCO_3^-, which is required for the HCO_3^--dependent fatty acid biosynthesis. Expression of FolC compensated for the observed CO_2-dependent decrease of intracellular follate levels.

Conclusions: The identification of putative pneumococcal transmission factors identified in this study could aid the design of intervention strategies to prevent acquisition of novel pneumococcal strains by the human host. It is foreseen that application of Tn-seq to conditions that reflect other relevant aspects of human transmission will identify more pneumococcal transmission factors.

O028

Ecology of chlorophyll-*d* containing cyanobacteria

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Most cyanobacteria employ chlorophyll (Chl) *a* and phycobiliproteins as major light harvesting pigments in their oxygenic photosynthesis. In contrast, cyanobacterial prochlorophytes use Chl *a* in combination with either Chl *b* (*Prochloron* and *Prochlorothrix*) or special divinyl derivatives of Chl *a* (*Prochlorococcus*) as major pigments. Yet other cyanobacteria have expanded their access to light energy via the use of near infrared (NIR) absorbing Chl *a* antennas associated with their photosystems, e.g. in *Arthrospira platensis* absorbing around 740 nm), or via the recently discovered Chl *f* absorbing up to 760 nm in an enrichment culture of filamentous cyanobacteria (Chen *et al.* 2010). However, the most spectacular spectral modification is found in the cyanobacterium *Acaryochloris marina* (Miyashita *et al.* 1996, 2003), which has almost completely exchanged its Chl *a* with Chl *d* absorbing maximally ~~712-718 nm *in vivo*. So far about 7 isolates of *A. marina* and the recently described *Candidatus* Acaryochloris bahamienis (Lopez-Legentil *et al.* 2011) are the only known organisms driving oxygenic photosynthesis with Chl *d*. While detailed ultrastructural, genetic and biophysical investigations of this unique phototroph have been reported, much less is known about the ecology and natural habitats of Chl *d*-containing cyanobacteria (Kühl *et al.* 2005). Since its first isolation from a didemnid ascidian, *A. marina* has been found in many different habitats and climate zones ranging from tropical coral reefs to continental Antartica and lakes in the Andes (Behrendt *et al.* 2011, Li *et al.* 2012), and pigment analysis of environmental sample has shown widespread occurrence of Chl *d* (Kashiyama *et al.* 2008). Thus NIR-driven oxygenic photosynthesis could be more widespread and important in particular environments. Judged from our findings, a common trait of *A. marina* in its natural habitats seems to be colonization of surfaces and growth in biofilms, where visible wavelengths are depleted but sufficient NIR prevails (Kühl *et al.* 2005, Larkum and Kühl 2005, Behrendt *et al.* 2011, 2012). Here I give an overview of our current knowledge of the ecology of Chl *d*-containing bacteria, including a description of their microhabitats, new results on their biofilm growth mode and information on a new isolate obtained from a new habitat.

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References:

1. Behrendt L, Larkum AWD, Trampe E, et al. Microbial diversity of biofilm communities in microniches associated with the didemnid ascidian Lissoclinum patella. ISME Journal. 2012 (online publ. Dec 1, 2011 doi:10.1038/ismej.2011.181).
2. Behrendt L, Larkum AWD, Norman A, et al. Endolithic chlorophyll d-containing phototrophs. ISME Journal. 2011;5:1072-6.
3. Chen M. Schliep M, Willows R, et al. A red-shifted chlorophyll. Science. 2010;329:1318-9.
4. Kashiyama Y, Miyashita H, et al. Evidence for global chlorophyll d. Science. 2008;321: 658.
5. Kühl M, Chen M, Larkum AWD. Biology of the Chlorophyll d-containing cyanobacterium Acaryochloris marina. In: J. Seckbach (ed.), Extremophilic algae, cyanobacteria and non-photosynthetic protists: From prokaryotes to astrobiology. 2007. Springer Berlin, pp. 101-23.
6. Kühl M, Chen M, Ralph PJ, et al. A niche for cyanobacteria containing chlorophyll d. Nature. 2005;433:820.
7. Larkum AWD, Kühl M. Chlorophyll d: the puzzle resolved. Trends in Plant Science. 2005;10:355-7.
8. Li Y, Larkum AWD, Schlie, M, et al. Newly isolated Chl d-containing cyanobacteria. Proceedings of 15th International Congress on Photosynthesis, Beijing, China 2010 (IN PRESS).
9. López-Legentil S, Song B, Bosch M, et al. Cyanobacterial Diversity and a New Acaryochloris-Like Symbiont from Bahamian Sea-Squirts. PLoS. 2011;ONE 6:e23938.
10. Miyashita H, Ikemoto H, Kurano N, et al. Chlorophyll d as a major pigment. Nature. 1996;383:402.
11. Miyashita H, Ikemoto H, Kurano N, et al. Acaryochloris marina gen.et sp. nov. (cyanobacteria), an oxygenic photosynthetic prokaryote containing Chl d as a major pigment. Journal of Phycology. 2003;39:1247-53.

O029

Redirecting intermediary metabolism of Synechocystis PCC 6803 for sustainable biofuel production

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The photanol concept has been introduced to refer to an approach to contribute to solving the problem of making a sustainable and renewable source of energy available that would also alleviate the problem of increasing CO_2 levels in the atmosphere.[1] The concept refers to the strategy that a range of low-molecular weight, high energy-density carriers ('alcohols'), could be formed from CO_2, water

and sunlight directly, by using genetically engineered cyanobacteria as a catalyst. Genetic engineering of these latter organisms may enable the introduction of a fermentative pathway that could lead to the conversion of central metabolic intermediates of the cyanobacteria (i.e. from the Calvin-Benson cycle, from glycolysis, etc.). The result is the formation of a metabolic chimera that can catalyze the above mentioned (overall) reaction.

Meanwhile for several end-products of fermentation pathways proof of principle for this approach has already been provided.[2-4] We are focusing efforts on the production of lactic acid, which can be achieved through integration – under proper promoter control – of a lactic acid dehydrogenase. Nevertheless, within this boundary condition further options are to make either D- or L-lactate, and to use either NADPH or NADH as the source of electrons for the conversion of pyruvic acid. Even then, differences are observed in rate and level of lactic acid production when Lactate-dehydrogenase enzymes are selected from different sources. Availability of reducing equivalents can be guided by proper choice of cofactor dependence of the dehydrogenase and/or by additional cloning of a (soluble) transhydrogenase.[5]

Co-expression of an NADH-dependent Lactate-dehydrogenase enzyme (LDH) and a soluble transhydrogenase (STH) enzyme leads to a higher lactate production rate and -yield, when compared to the corresponding single lactate dehydrogenase mutant and when compared to results of previous investigations by others. The expression of a transhydrogenase alone is unbeneficial and a mutant carrying such a gene will be outcompeted by cells having a disrupted transhydrogenase gene. The addition of a LDH can rescue this phenotype and can prevent this reversion.

References

1. Angermayr et al. (2009) Energy biotechnology with cyanobacteria. Curr. Op. Biotec. 20:3
2. Deng and Coleman (1999) Ethanol Synthesis by genetic engineering in Cyanobacteria. Appl. Envi. Microbiol. 65:2
3. Atzumi et al. (2009). Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. 27:12
4. Lan and Liao (2011) Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. 13:4
5. Niederholtmeyer et al. (2010) Engineering synthesis and export of hydrophilic products from cyanobacteria. Appl. Envi. Microbiol. 76:11

O030

Energy budget and light utilization efficiency in photosynthetic microbial mats

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Photosynthetic microbial mats are amongst the most investigated microbial ecosystems, yet the overall energy efficiency of such purely microbial ecosystems is largely unexplored. Here, we focus on light energy budget and the efficiency with which light as the primary energy source is utilized and converted to chemical energy in three photosynthetic mats originating from locations with different environmental characteristics. We followed the fate of light energy in the mats using microsensor-based approach. We used microsensors for irradiance, temperature and O_2 to measure, respectively, the rates of light absorption, heat production and photosynthetic energy conservation in the system. Subsequently, we divided the respective energy fluxes with the flux of absorbed light energy to calculate the efficiencies of light energy conservation and heat dissipation as a function of the absorbed light flux. These functional measurements were supplemented with pigment analysis and microscopy to characterize the influence of structural parameters on the light energy budget in the different mats, with a special focus on the efficiency of light energy conservation by photosynthesis. The euphotic zones in the three studied mats differed in their phototrophic community structure, pigment concentrations and thickness. In all mats, < 1% of the absorbed light energy was conserved via photosynthesis at high incident irradiance, while the rest was dissipated as heat. Under light limiting conditions, the photosynthetic efficiency reached a maximum, which varied among the studied mats between 4.5 and 16.2% and was significantly lower than the theoretical maximum of 27.7%. The maximum efficiency correlated linearly with the light attenuation coefficient and photopigment concentration in the euphotic zone. Higher photosynthetic efficiency was found in mats with a thinner and more densely populated euphotic zone. Microbial mats exhibit a lower photosynthetic efficiency compared to ecosystems with a more open canopy-like organization of photosynthetic elements, where light propagation is not hindered to the same extent by photosynthetically inactive components; such components contributed about 40-80% to light absorption in the investigated microbial mats, which is in a similar range as in oceanic planktonic systems.

O031

Transition between oxygenic and anoxygenic photosynthesis in cyanobacteria from the Frasassi sulfidic springs

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Sulfidic springs emerging from the Frasassi cave system, Italy, harbor thick biofilms dominated by cyanobacteria and filamentous colorless sulfur bacteria. The sulfide

concentrations reach up to 600 μM in the overlying spring water and up to 1 mM in the underlying sediment. *In situ* oxygen, H_2S and pH microsensor measurements show that, depending on the incident light intensity, the dominating cyanobacteria shift between anoxygenic and oxygenic photosynthesis, or perform both simultaneously. Under low light conditions (incident intensity below ~200 μmol photons m^{-2} s^{-1}), only anoxygenic photosynthesis was performed. This activity decreased sulfide concentrations in the biofilm, but was not sufficient to counteract the diffusive transport of sulfide from the overlying water and the underlying sediment to deplete sulfide below a threshold value of ~200 μM, which would allow a switch to oxygenic photosynthesis. At higher incident light intensities, when the sulfide in the biofilm was depleted below the inhibitory value, oxygen production recovered and was performed simultaneously with anoxygenic photosynthesis. The rate of oxygenic photosynthesis increased with decreasing sulfide concentrations in the biofilm and was maximal when sulfide was completely depleted. Furthermore, the recovery time increased with the increasing exposure to sulfide in the dark, with only light adapted cyanobacteria being able to immediately shift to oxygenic photosynthesis after sulfide depletion below ~200 μM. We hypothesize that the regulatory effect of sulfide and light on the switch between oxygenic and anoxygen photosynthesis in the studied cyanobacteria can be explained by the combination of three factors: (i) toxicity of H_2S to photosystem II, (ii) the redox state of the plastoquinone pool, which is, due to the fact that the plastoquinone is shared among several different energy generating processes in the electron transport chain, regulated kinetically by the availability of external electron donors (organic matter, sulfide), acceptors (oxygen, DIC) and light, and (iii) the expression level of sulfide quinone reductase (SQR), which facilitates electron transfer from sulfide to the electron transport chain at the plastoquinone site.

O032

Chlamydia psittaci, causative agent of avian chlamydiosis and human psittacosis: risk assessment and management

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Chlamydia psittaci causes respiratory disease in poultry and can be transmitted to humans. Human infections are underestimated, as demonstrated by a prevalence study in a human population in contact with domesticated birds. Outbreaks of psittacosis in poultry workers indicate the need for higher awareness and efficient risk assessment and management. Relevant information and basic suggestions on how to assess and manage the risk of psittacosis in poultry processing plants are made based on a classical four-step approach. Collective and personal protective measures, as well as the role of occupational medicine are discussed. Complete eradication is difficult to achieve. Ventilation, cleaning, hand hygiene and personal protective equipment are the most important protective measures to limit and control exposure to *Chlamydia psittaci*. Adequate information, communication and health surveillance belong to the responsibilities of the occupational physician. Future challenges lay in the rigorous reporting of infections in both poultry and poultry workers, and in the development of an avian *Chlamydia psittaci* vaccine to reduce the zoonotic risk.

O033

Diagnosing Psittacosis and typing of *Chlamydia psittaci*

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Accurate diagnostic options are needed to facilitate early detection of psittacosis. Serologic tests were the mainstays of the diagnosis in the last few decades. Micro-immunofluorescence (MIF), complement fixation (CF) and ELISA tests are commonly used. However, serologic tests have problems with sensitivity and specificity. In general, serologic testing has to be performed on two serum samples. Serology provides only a retrospective diagnosis. Real-time PCR for detection of *Chlamydia psittaci* seems very specific, fast and sensitive if applied to an appropriate sample (sputum/broncho-alveolar lavage). A drawback for use of PCR is the need for deep respiratory samples (sputum or broncho-alveolar lavage (BAL) fluids). In general, patients with pneumonia do often not expectorate sputum. This can still remain an obstacle for correct diagnosis. In these cases BAL could be considered. From an epidemiological point of view, real-time PCR can aid in obtaining more precise incidence and prevalence numbers and monitoring of the frequency of this disease will therefore be more straightforward.
Formerly, serotyping was the standard for typing of *C. psittaci* strains. In the Netherlands culture of *C. psittaci* in the diagnostic setting is abandoned. Mainly because of biosafety reasons. Genotyping of the outer membrane protein is currently accepted as a more accurate and sufficient substitute for serotyping of *C. psittaci*. It can help to identify avian sources of human psittacosis cases and monitor the incidence of the different genotypes to

infer the most likely avian sources as most genotypes are more or less associated with certain bird species. Probably genotypes A&B are the most prevalent genotypes in Human infections. A newly developed OmpA based genotyping method which has been used on human clinical samples will be presented at the NVMM meeting. Attempts should be made in the near future to develop genotyping methods with more discriminating power. Application of these methods directly on clinical samples will be the challenging issue. Genotyping of avian and human strains will identify the avian sources that are the most relevant in view of the zoonotic potential. Currently, everyone who buys a bird is at risk of ending up with an infected bird and a subsequent zoonotic *C. psittaci* infection. Although notification of psittacosis has been included in the 'Wet publieke gezondheid' (dutch) and protocols have been issued on how to deal with this disease, outbreak management among birds relies completely on cooperative bird owners and vendors. Besides pet bird contact, psittacosis can also be acquired from environmental birds. The impact of this potentially large *C. psittaci* reservoir in the environment as (in)direct reservoir for zoonotic disease has still to be determined. In my opinion, from a public health view the first objective is to implement a surveillance of human *C. psittaci* infections which relies mainly on PCR with subsequent genotyping to monitor changes and to infer the most probable avian sources. To my knowledge there is no such surveillance anywhere in the world but would be feasible in a country as the Netherlands with a good microbiological infrastructure and cooperation with public health officials.

O034

Veterinary aspects and control of psittacosis

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Psittacosis is a bird borne zoonosis that is caused by the intracellular bacterium *Chlamydophila psittaci*. The diseases name relates to the fact that psittacine birds are by far most frequently implicated in human cases. However, not only psittacines may carry the bacterium. A wide range of avian species has been shown to harbour the micro-organism, with psittacines and pigeons exhibiting highest infection rates. Seven genotypes of *C. psittaci* can be distinguished, each of which tends to be associated with certain avian hosts.

Taking into account the broad host range of *C. psittaci* in birds, veterinarians refer to avian infections as avian chlamydiosis' rather than psittacosis. *C. psittaci* causes systemic infections in birds with a variety of symptoms. Latent infections without clinical signs and intermittent shedding are nonetheless widespread. These can be triggered by administration of sub-therapeutic doses of antibiotics which is thought to be commonplace in bird trade. Avian chlamydiosis is usually treated with tetracyclines. Treatment sometimes faces practical problems in that parenteral administration may cause muscle necroses, thus prohibiting injection of smaller bird species and oral administration by medicated feeds or drinking water may fail to produce therapeutic plasma levels due to avoidance. Several laboratory tests are available for the diagnosis of avian chlamydiosis. Official testing in the course of veterinary outbreak investigations relies on PCR.

Avian chlamydiosis is a notifiable disease under the Animal Health and Welfare Act. Veterinarians and bird owners are required notify the Dutch Food and Consumer Product Safety Authority (NVWA) when avian chlamydiosis is suspected or diagnosed. Notification instigates an official investigation by NVWA with sampling of affected/suspected birds. If avian chlamydiosis is confirmed, affected birds are treated mandatorily for the course of six weeks and quarantined. Quarantine is not lifted until the birds in question have been shown to cease shedding two weeks after the administration of the last dose of antibiotics. Psittacosis in human patients is notifiable too. Provided that notification criteria are met, practitioners inform their Municipal Health Service (GGD). Upon notification, GGD commences a source finding investigation. When birds are implicated in the particular case, GGD can request NVWA to investigate the birds in question. Should the animals be shown to shed *C. psittaci*, NVWA proceeds as outlined.

O035

Multi locus sequence typing of *Chlamydia psittaci* and *Chlamydia pecorum* from birds and mammals reveals an association between *Chlamydia* genotypes and host species

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Chlamydia comprises a group of obligate intracellular bacterial parasites responsible for a variety of diseases in humans and animals, including several zoonoses. *Chlamydia psittaci*, causing zoonotic pneumonia in humans, is usually hosted by birds. *Chlamydia pecorum* is the causative agent of reproductive and ocular disease in several animal hosts including koalas, sheep, cattle and goats. *Chlamydia* isolates are commonly typed on bases of OmpA variety. Previously, we showed using multi-locus

sequence typing (MLST) an association between *C. psittaci* genotypes with host species (Pannekoek et al PLoS One. 2010 Dec 2;5(12):e14179). Here, we MLST to asses the genotype of *C. psittaci* and *C. pecorum* isolates from different host, including mammals. In total, 53 *Chlamydia* isolates were analyzed, including 28 *C. psittaci* isolates (8 from pigeons, 10 from psittacines, 6 from ducks, and one from poultry, ibis, and human) and 15 *C. pecorum* isolates (8 from cows, 6 from pigs and one from sheep). In addition, 10 *Chlamydia* isolates suspected to be *C. psittaci* and isolated from cows (7), one from rabbit, rat, and sheep (one from each) were assessed by MLST. Results showed more diversity among the *Chlamydia* isolates when typed by MLST than by OmpA typing. Cluster analyses utilizing the Neighbour-Joining algorithm with the maximum composite likelihood model of concatenated sequences of 7 housekeeping fragments showed that *C. psittaci* genotypes appeared to be associated with bird host species as previously described by us. The *C. psittaci* from cows and from rabbit and rat grouped together with those form psittacines and thus were confirmed to be *C. psittaci*. The isolates from poultry, ibis, sheep and human grouped together with those from ducks. The *C. pecorum* isolates formed two main groups, one consisting of all but one *C. pecorum* isolates from cows and the one *C. pecorum* isolate from sheep and one consisting of all isolates from pigs and one from a cow. In conclusion, MLST has a higher discriminating power than OmpA typing. The results show that *C. psittaci* infections are not solely confined to bird species but can also be isolated from mammals, suggesting host species jumps. Genotypes of *C. pecorum* are associated with host species.

O036

The challenges of serological prediction of chronic Q fever

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Introduction: After primary infection with *Coxiella burnetii*, 1-5% of patients develop chronic Q fever. Endocarditis and infection of a vascular prosthesis or aortic aneurysm are the most important manifestations. Chronic Q fever has a morbidity and mortality of> 60% when left untreated. PCR and culture on blood have low sensitivity for detection of chronic Q fever. Hence diagnosis relies mainly on serologic tests, of which immunofluorescence assay (IFA) is the most commonly used. Cut-off titers for phase I IgG to detect chronic infection are matter of debate, but are estimated between 1:800 and 1:1600. To improve diagnostic work-up, we studied the serological profiles in patients with established chronic Q fever.

Methods: We selected all patients included until September 2011 in the Dutch National Database of Chronic Q Fever Patients. According to Dutch consensus, patients were categorized as proven, probable or possible chronic Q fever. This classification ranks the probability of having chronic Q fever based on PCR results, serology, clinical parameters, imaging studies and pathology. We examined phase I IgG antibody titers (IFA) at time of positive blood PCR, at diagnosis, and at peak levels and compared these between the three chronic Q fever subgroups.

Results: We evaluated 200 patients, of whom 56 (28.0%) had possible chronic Q fever, 51 (25.5%) probable chronic Q fever, and 93 (46.5%) proven chronic Q fever. Of the patients with proven chronic Q fever, 52 patients (55.9%) had a positive *C. burnetii* PCR in blood, 10 (10.8%) in tissue and 13 (14.0%) in both blood and tissue. Logistic regression analysis demonstrated a significant rise in probability of positive PCR in case of each increasing phase I IgG titre, using a binary dilution scale (Odds Ratio (OR) 1.35, 95% confidence interval 1.02-1.77, p = 0.033) Median phase I IgG titers at time of diagnosis and peak titer in patients with proven chronic Q fever were significantly higher (both 1:8192, p < 0.05), compared to patients with probable (1:2048 and 1:4096, respectively) and possible chronic Q fever (both 1:2048). The positive predictive value (PPV) for proven chronic Q fever was 62.2%, 66.7%, 76.5%, 86.2%, 93.6% and 94.4% for phase I IgG titers of respectively 1:1024, 1:2048, 1:4096, 1:8192, 1:16384, ≥ 1:32786. Sensitivity was 97.8%, 94.6%, 80.6%, 60.2%, 47.3% and 25.8% for phase I IgG titers of respectively 1:1024, 1:2048, 1:4096, 1:8192, 1:16384, ≥ 1:32786.

Conclusion: Our study shows that high phase I IgG titers are strongly associated with proven chronic Q fever, especially when exceeding 1:4096 (PPV> 86%). We found positive association between height of phase I IgG titer and the likelihood of a positive PCR in blood samples. Due to low sensitivity (< 60%) of phase I IgG titers of> 1:4096 and high morbidity and mortality of untreated chronic Q fever, increasing the current diagnostic phase I IgG cut-off is not recommended. Our study emphasizes that, in case

of chronic Q fever, serology is not a diagnostic tool on its own. Therefore, serologic results should, in the absence of a positive PCR, be interpreted in combination with clinical parameters.

O037

Discovery of a new azole resistance mechanism in *Aspergillus fumigatus* through whole genome sequencing and sexual crossing

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Introduction: azole treatment is the primary antifungal therapy for patients with disease caused by *Aspergillus fumigatus*. Although *A. fumigatus* is generally susceptible to this class of antifungal agents, acquired resistance is increasingly being reported. The most common mechanisms of resistance in *A. fumigatus* are modifications of the *cyp51A* gene, encoding the target for azoles. In addition, alterations of the promoter region have been described, resulting in an elevated expression of the gene. However, not all resistant isolates show such alterations, indicating that other yet unknown mechanisms also play a role. To date, no other gene alterations have been identified as underlying resistance mechanism in clinical *A. fumigatus* isolates. We investigated patient isolates with the purpose to find a new mechanism causing azole resistance.

Methods: our patient was a 21 year-old man with chronic granulomatous disease. Due to a pulmonary infection caused by *A. fumigatus*, he was treated with multiple courses of antifungal therapy. We obtained four sequential *A. fumigatus* isolates from the patient; the first two isolates were susceptible to azole antifungals but the last two isolates showed resistance. Despite antifungal treatment it was not possible to eliminate the fungus and the patient ultimately died from his pulmonary infection.

Because this set of isolates was isogenic and did not show any mutations in *cyp51A*, we had a unique opportunity to study the development of the underlying resistance mechanism. Our strategy included whole genome sequencing (Illumina technology) of the last sensitive isolate and the first resistant isolate, to screen for potential resistance mutations. Assembly of the complete genome sequence was done using Maq and only non-synonymous mutations in the resistant isolate relative to the sensitive isolate and both non-resistant reference isolates Af293 and A1163 were selected. Mutations were confirmed by conventional PCR and Sanger sequencing. To elucidate which (combination of) mutations was the cause of resistance, sexual crosses and gene replacement experiments were performed.

Results: Whole genome sequencing and subsequent confirmation revealed six mutations. The progeny of the sexual cross were screened for the presence of these six mutations. All mutations were present in part of the susceptible as well as in part of the resistant progeny, except for the mutation in the *hapE* gene which was present in all resistant progeny but never in susceptible progeny. This strongly suggested that this mutation caused the observed resistance phenotype. Gene replacement experiments, in which wildtype *hapE* was exchanged for the mutated gene, provided the final evidence that this mutation was indeed responsible for the azole resistant phenotype.

Conclusion: combining a unique comparative genomic and genetic approach, we have shown that a single base substitution in HapE is responsible for azole resistance in *A. fumigatus*. Importantly, this is the first non-*cyp51A*-mediated azole resistance mechanism identified in clinical *A. fumigatus* isolates to date. The discovery may help understand alternate pathways of azole resistance in *A. fumigatus* with implications for the molecular diagnosis of resistance and drug discovery.

O038

Mannitol-1-phosphate dehydrogenase is essential for the development of extreme stress resistant fungal ascospores

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Ascospores of Neosartorya fischeri exhibit extreme stress resistance. They survive extreme drought (down to 0.5% relative humidity), high temperature (20 minutes at 85°C), high pressure (6000 Bar) and various chemical stresses (e.g. pH and salt stress). The spores are constitutively dormant and can survive several years in a dormant state. Exit of dormancy, and subsequent germination, can be realized by a short "heat flash"at 85°C. While much research has been performed on the characterization of spores, not a lot is known about the process of ascospore development. During maturation ascospores become more heat resistant; this is accompanied with an increase of micro-viscosity and an increase of compatible solutes (e.g. trehalose). A remarkable observation is the high concentration of mannitol in young spores, which slowly decreases during maturation of the spores. To evaluate the role of mannitol in development of ascospores, two genes involved in the mannitol metabolism (mannitol-1-phosphate dehydrogenase (MPD) and mannitol dehydrogenase (MDH)), are deleted within N. fischeri. The MPD mutant is not

producing fully developed ascospores, while the formation of ascomata and asci is not affected.
Within conidia (asexual spores), mannitol is thought to play a role in stress resistance and dormancy. We hypothesize a different role of mannitol. High mannitol concentration could result in an osmotic pressure, attracting water and nutrients to the ascocarp needed for the formation of functional spores. Ongoing research on the promoter of the two mannitol synthesis genes and qPCR will give us more information about when and where MPD is transcribed.

O039

Effects of corticosteroids on submerged growth of *Aspergillus fumigatus* and *Aspergillus niger*

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Introduction: Corticosteroid treatment is associated with invasive aspergillosis. Corticosteroids do not only have an immune suppressive effect on the host, but also an effect on hyphal growth of *Aspergillus* strains. In submerged culture, *Aspergillus* strains form microcolonies. Their diameter correlates with hyphal growth. The size of microcolony diameters can be assessed with Complex Object Parametric Analyzer and Sorter (COPAS), measuring time of flight (TOF).
Aim: We investigated the effects of the corticosteroids hydrocortisone, methylprednisolone and dexamethasone in physiological concentrations on microcolony diameter size.
Methods: *Aspergillus fumigatus* AF293 and *Aspergillus niger* N402 were incubated in minimal medium with 0.5% yeast extract, 0.2% casamino acids, and 2% glucose at 200 RPM with and without the corticosteroids hydrocortisone (1 μM), dexamethasone (0.5 μM), and methylprednisolone (0.5 μM). A minimum of 500 microcolonies were assessed for size (TOF) using COPAS Plus (Union Biometrica, Holliston, MA). Sorting parameters were set in such a way that clusters of colonies or debris were excluded from the analysis. Pooled data analysis of at least three biological replicates is presented.
Results: Incubation with both hydrocortisone and methylprednisolone resulted in significantly larger microcolony diameters of *Aspergillus fumigatus* AF293 (mean difference TOF to control 85 SEM. 1.1, and mean difference 61 SEM 4.5, respectively), and smaller diameters of *Aspergillus niger* N402 compared to control medium without corticosteroids (mean difference TOF to control -571 SEM 28, and mean difference -255 SEM 21, respectively). Dexamethasone did neither influence microcolony diameter size of *Aspergillus fumigatus* (mean difference TOF to control 1.4 standard error 6.6), nor of *Aspergillus niger* (mean difference TOF to control 29.3 standard error 20.6).
Conclusion: *Aspergillus fumigatus* growth is influenced by hydrocortisone and methylprednisolone, but not by dexamethasone. Future studies should assess whether treatment with dexamethasone reduces the risk on invasive aspergillosis compared to methylprednisolone.

O040

Identification of moulds with Matrix Assisted Laser Desorption and Ionization-Time of Flight Mass Spectrometry

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Introduction: Rapid and correct identification of fungal infections enables the physician to start appropriate treatment early in the course of the infection. Matrix Assisted Laser Desorption and Ionization- Time of Flight Mass Spectrometry (MALDI-TOF MS) is a routinely applied identification method for bacteria and yeasts and seem to be promising for identification of fungi. The aim of this study is to identify clinically important moulds rapidly by MALDI-TOF MS.
Methods: Moulds included in this study encompassed Scopulariopsis species, Absidia species, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Trichophyton terrestre* and *Trichophyton rubrum*. Isolates were obtained from routine diagnostics using growth on Dermatophyte Test Medium, DTM, growth at 42C, urease activity and morphological characteristics. Isolates were cultured on Sabouraud agar, at 27 and 35 degrees and, if appropriate, on DTM. All specimens were analyzed in duplo at day two, three and seven. Five different pretreatment extraction methods were applied;
A: standard LUMC extraction method, as described by Veloo *et al*. Clinical Microbiology and Infection 2011;
B: increased volume of solvent, water and ethanol, longer centrifugation time (10 instead of two minutes) and 10 mcl formic acid and acetonitrile (method A 25 mcl) to dissolve pellet;
C: like protocol B, but with three cycles of 60 seconds beads beating before centrifugation.
D: solvent like A, further like B;
E: direct inoculation of target plate suspended in water followed by drying and ethanol treatment.
After pretreatment, samples were inoculated on the target plate and matrix was added.
Mass spectra were retrieved with MALDI-TOF MS (Bruker Daltonik, FlexControl software version 3.0, Biotyper software nr 3995-4111) and compared to a database containing two *A. flavus*, six *A. fumigatus,* one *A. versicolor* and two *T. rubrum* spectra. The database did not contain spectra of Scopulariopsis species, Absidia species and T. terrestre.

Results: Method B yielded mass spectra for three out of six samples on day two and for five out of seven samples on day three and seven. This method identified *A. fumigatus* and *T. rubrum* at day three and *A. flavus* and *A. fumigatus* at day seven at species level. Method D yielded mass spectra for six out of seven samples on day three and for two out of three samples on day seven. Method D correctly named *A. flavus* and *A. fumigatus* on day three and *A. flavus* on day seven, but no sufficient correlation to identify. The mass spectra of the different days varied within the same samples.

Methods A, C and E yielded less promising results. These methods resulted in less adequate mass spectra compared to method B and D. However, no incorrect identification was given by any method.

Conclusion: The identification of moulds using MALDI-TOF is promising, but needs standardization of culture media and incubation conditions. The spectra retrieved on different days are variable, suggesting a database of young and old moulds would be of value.

Best results were obtained after 3 and 7 days using a large amount of solvent, 10 minutes of centrifugation and 10 mcl formic acid and acetonitrile to dissolve the pellet, method B.

O041

Aspergillus fumigatus mycovirus infection is not dependent on the genetic up-make of the host

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Introduction: Mycoviruses are viruses that selectively infect fungi and are ubiquitous in all major groups of filamentous fungi. Most mycoviruses have dsRNA genomes and replicate cytoplasmatically. Although most of the mycoviruses cause cryptic infections, mycoviruses are of scientific interest since some of these viruses can cause fungal hypovirulence such as reduced growth rate, altered pigmentation and sporulation. Viruses are considered to be host-specific, but within each host some individuals are more prone to develop a viral infection than others. Therefore, we determined if the genetic make-up of *Aspergillus fumigatus* was correlated to the presence of a mycovirus.

Materials and methods: A collection of 112 clinical *A. fumigatus* isolates from the Erasmus MC, Rotterdam, the Netherlands was screened for mycovirus presence by isolating dsRNA from fresh lyophilized mycelial cultures using a Trizol/chloroform method. To determine genetic relatedness of *A. fumigatus* the cell surface protein (CSP) gene was typed by sequencing.

Results: Of the 112 clinical *A. fumigatus* isolates 16 (14.3%) contained dsRNAs. The *A. fumigatus* collection could be divided into 12 different CSP types, indicating that the collection used was of heterogenous origin. *A. fumigatus* isolates which contained dsRNA mycoviruses had similar CSP types as non-infected isolates. In both cases, the CSP types 1, 2, 3 and 4 were the most prevalent which was comparable to the CSP types observed in other Dutch collections.

Conclusion: Mycovirus infection is not related to a specific genetic *A. fumigatus* lineage.

O042

Filamentous fungi in cystic fibrosis patients in the Netherlands

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Objectives: The clinical significance of fungal infections in cystic fibrosis (CF) is poorly understood. Deterioration of lung function in these patients is multi causal. In addition, there is evidence of an increasing prevalence of filamentous fungi in sputum but the reported results vary widely between countries and centres (9-56%). Little is known about the epidemiology, genotypes and resistance patterns of filamentous fungi in Dutch patients with CF. Here we report results of a prospective study of fungal colonization in CF patients in the Netherlands.

Methods: Standardized routine sputum samples were collected in four CF.

Centres. All samples were cultured on mould-selective plates and incubated at two temperatures. All filamentous fungi were stored centrally. After initial morphologic identification, molecular confirmation using AFLP and ITS + D1/D2 sequencing was performed. All *Aspergillus fumigatus* isolates were additionally screened for presence of the most common mutations in the CYP51 gene conferring azole resistance.

Results: In the period May 2010 - September 2011, 1768 samples from 653 patients containing 105 different species of filamentous fungi. The main species found was *A. fumigatus* (n = 1072). In addition, other potentially pathogenic fungi

were isolated. These included: *Pseudallescheria/Scedosporium complex* (n = 59), *A. terreus* (n = 15) and *Exophiala dermatitidis* (n = 31) Next to these, a wide range of other filamentous fungi with thus far unknown pathogenic potential were isolated. This group includes other *Aspergillus* sp. and a wide variety of *Penicillium* sp. (total isolates 212) Interestingly we also report the first isolation of *Geosmithia argillaceae* (n = 8) in Dutch CF patients. A total of 5.5% of the *A. fumigatus* isolates were azole resistant and dominantly harbour the TR/L98H mutation. A single G54W was identified while no other mutations at positions G138 or M220 responsible for azole resistance where recovered.

Conclusion: These results suggest that the Dutch epidemiology of filamentous fungi in CF patients is comparable to other countries. The main fungus was *A. fumigatus*, but also other fungal species such as *Scedosporium* sp. and *Exophiala* sp. were identified including the newly described species *Geosmithia argillaceae*. In addition 5.5% of *A. fumigatus* isolates appeared to contain mutations reported to confer azole resistance. These are the first epidemiological data on azole resistance in *A. fumigatus* from CF patients in the Netherlands.

O043

Active polyomavirus infection characterizes trichodysplasia spinulosa

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Introduction: A new polyomavirus was identified in a patient with trichodysplasia spinulosa (TS), a rare skin disease of immunocompromised patients characterized by the growth of keratinous spines on the face from dysmorphic hair follicles overgrown by inner root sheath cells. Seroepidemiological studies indicate that the TS-associated polyomavirus (TSV or TSPyV) is ubiquitous and latently infects 70% of the healthy individuals. To corroborate the relationship between TSV infection and TS disease, we analyzed the presence, load, expression and localization of TSV infection in TS patients and in controls.

Method: TS lesional and non-lesional skin samples were obtained from 11 TS patients retrieved through a PubMed search. These samples were analyzed for the presence and load of TSV DNA with quantitative PCR, and for expression and localization of viral VP1 protein with immunofluorescence. The results were compared with those obtained from 249 healthy controls.

Results: Detection of TSV DNA was significantly associated with TS disease ($p < 0.001$), with 100% positivity of the lesional and 2% of the control samples. Quantification revealed high TSV DNA loads in the lesional samples ($\approx 10^6$ copies/cell), and low viral loads in the occasionally TSV-positive non-lesional and control samples ($< 10^2$ copies/cell). TSV VP1 protein expression was detected only in lesional TS samples, which was found to be restricted to the nuclei of inner root sheath cells over-expressing trichohyalin.

Conclusion: The high prevalence and load of TSV DNA only in the TS lesions, and the abundant expression of TSV protein in the affected hair follicle cells demonstrate a tight relation between TSV infection and TS disease, and indicate involvement of active TSV infection in the pathogenesis of TS.

O044

The paradox of maternal immunity as a risk factor for congenital cytomegalovirus infection: a population-based prediction model

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Introduction: Maternal immunity to cytomegalovirus (CMV) provides substantial protection against severe congenital CMV disease. Paradoxically, the prevalence of congenital CMV increases with CMV seroprevalence in the underlying population. The objective of our study was to quantify the contribution of non-primary maternal CMV infection on the disease burden of congenital CMV as a function of the seroprevalence in the population.

Methods: A population-based prediction model was developed and applied, estimating the proportion of children with congenital CMV and CMV-related sequelae attributable to non-primary maternal infection, with CMV seroprevalence in the population as independent variable.

Results: Both the proportion of newborns with congenital CMV and the proportion of newborns with sequeale, attributable to non-primary maternal infections increased with CMV seroprevalence in the underlying population. These proportions were up to 96% (95% CI 88-99%) and 89% (95% CI 26-97%), respectively, in populations with seroprevalence of 95%.Furthermore, seropositive pregnant

women were found to be at higher risk of having a congenitally infected newborn than seronegative pregnant women, for all population CMV seroprevalence values. In contrast, seropositive pregnant women were at lower risk of having a newborn sequelae related to congenital CMV than seronegative pregnant women.

Conclusions: Our data stress the impact of non-primary congenital CMV infection on the disease burden of congenital CMV, among all (sub)populations. Awareness of the risk for seropositive women of having a newborn with CMV-related sequelae will have significant consequences for preventive strategies including hygienic counseling, maternal serological screening, and immunization studies.

O045

Binding of avian coronavirus spike proteins to host factors reflects virus tropism and pathogenicity

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Introduction: The avian coronavirus infectious bronchitis virus (IBV) is the most common causative agent of respiratory disease in poultry worldwide. In contrast to avian influenza A virus (IAV), IBV has a restricted host tropism, while both viruses depend on α2,3-linked sialic acids for their infection. Between IBV strains, however, the tissue tropism and pathogenicity can vary widely. As attachment of a virus to the host cell is the first step in determining the cell, tissue and host tropism, we here investigated whether the interaction between the viral attachment proteins and the host could explain these differences.

Methods: We used recombinant soluble viral attachment proteins to study their binding to avian tissues and glycans. In particular, spike S1 domains of IBV strains with diverse pathogenicity and the HA ectodomain of avian influenza virus H5N1 were generated by expressing them in a mammalian expression system. Binding to various chicken and goose tissues was analyzed by protein histochemistry. Glycan array analyses were performed in collaboration with the Consortium for Functional Glycomics.

Results: Our data showed that the S1 protein of the virulent IBV-M41 specifically bound to cilia and goblet cells of the chicken respiratory tract, in accordance with the reported sensitivity of these cells to IBV. While the binding specificity of HA for the chicken trachea and lung closely resembled that of M41-S1, its binding avidity was markedly higher. In accordance with IBVs narrow host range, M41-S1 did not bind epithelial cells of goose respiratory tract, in contrast to the HA protein of H5N1. The fine receptor specificity was further elucidated in the glycan array, in which HA bound with high avidity to a broad range of α2,3-linked sialylated glycans, while M41 S1 recognized only one particular α2,3-linked disialoside. When analyzing the binding of IBV-S1 proteins of the vaccine H120 and the non-virulent Beaudette strain we observed respectively low or no affinity for chicken trachea and lung epithelium, which reflects virus growth *in vivo*. Remarkably, S1 binding of the more divergent nephropathogenic IBV-B1648 required prior removal of sialic acids from the spike, and notable differences in cell and tissue binding preferences were observed.

Conclusion: In agreement with their different tropisms, the attachment proteins of IBV and IAV differed dramatically in avidity and specificity for avian tissues and glycans. Furthermore, the attachment patterns of IBV-S1 are in accordance with the reported IBV tropism and pathogenicity profiles. In conclusion, our recombinant S1 proteins are excellent tools for the further dissection of spike-host interactions and for elucidating parallels and differences between IBV strains, but also between other avian viruses, as demonstrated here by the HA protein binding of avian influenza virus H5N1.

O046

The use of MMP-8 and MMP-9 to assess disease severity in children with viral lower respiratory tract infections

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Introduction: Matrix metalloproteinases (MMPs) play an important role in respiratory inflammatory diseases, such as asthma and chronic obstructive pulmonary disease. We hypothesized that MMP-8 and MMP-9 may function as biological markers to assess disease severity in viral lower respiratory tract infections in children.

Methods: MMP-8 and MMP-9 mRNA expression levels in peripheral blood mononuclear cells (PBMCs) and granulocytes obtained in both the acute and recovery phase from 153 children with mild, moderate and severe viral lower respiratory tract infections were determined using real-time PCR. In addition, MMP-8 and MMP-9 concentrations in blood and nasopharyngeal specimens were determined during acute mild, moderate and severe infection and after recovery using ELISA. Furthermore, PBMCs and neutrophils obtained from healthy volunteers were stimulated with RSV, LPS (TLR4 agonist) and Pam3Cys (TLR2 agonist) *in vitro*.

Results: Disease severity of viral lower respiratory tract infections in children is associated with increased gene expression levels of MMP-8 and MMP-9 in both PBMCs and granulocytes and with MMP-8 plasma concentrations.

On the contrary, *in vitro* experiments showed that MMP-8 and MMP-9 mRNA and protein expression in PBMCs and granulocytes is not induced by stimulation with RSV, the most frequent detected virus in young children with viral lower respiratory tract infections.

Conclusion: Our data indicate that MMP-8 and MMP-9 expression levels in both PBMC and neutrophils are associated with viral lower respiratory tract infections disease severity. These observations justify future validation in independent prospective study cohorts of the usefulness of MMP-8 and MMP-9 as potential markers for disease severity in viral respiratory infections.

O047

Norovirus in hospitalized children: clinical, epidemiological and virological features

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Introduction: Noroviruses are the most common cause of acute viral gastro-enteritis worldwide. They have a large genetic variability and upsurges in the number of norovirus infections are thought to be related to emerging novel variants of existing genotypes. Noroviruses are highly infectious, and are able to cause large outbreaks especially associated with health care institutions. The development of molecular diagnostic techniques has facilitated the detection and characterization of noroviruses, however, the value of these techniques is not well established because of the lack of relating clinical and epidemiological data.

Methods: As part of a prospective study into gastro-intestinal infections in children hospitalized in the UMCG Beatrix Children's Hospital, we collected clinical data of all children of whom fecal samples were taken for the detection of 6 enteric viruses. We further characterized noroviruses by sequence analysis. Positive amplification as well as sequence analysis were related to clinical symptoms and patients characteristics. Sequence based typing results were combined with epidemiological data to give insight in transmission routes of norovirus within the hospital and to detect possible sources of nosocomial transmission. Also the relative viral load was determined to understand these transmissions.

Results: From October 2009 till December 2011, 802 patients were included with 1068 disease episodes. Norovirus was the second most frequently detected virus (134 episodes), only preceded by adenovirus (192 episodes). In 462 events, no enteric virus could be detected. Patients with norovirus were significantly older than the ones who had no virus detected (median 8.5 versus 3.0 months, $p < 0.003$), and had significantly more complaints of vomiting (52% versus 23.5%, $p < 0.001$) and diarrhea (77% versus 26%, $p < 0.001$). No association was detected between the amount of virus and the occurrence of symptoms. Genogroup II.4 2010 was the most frequently detected genogroup (25%), followed by genogroup II.b (19%) and genogroup II.4 2006b (15%). No differences were seen in patients' characteristics between the different genogroups, except for the presence of chronic underlying illness which was significantly more present in patients with norovirus genogroup II.4 2010 (76% versus 45% in genogroup II.b and 36% in genogroup II.4 2006b). Hospital acquired infection, defined as a first day of illness more than 5 days after admission, was present in 42% of the norovirus positive events.

Conclusion: Noroviruses are among the most frequently detected viruses in children hospitalized in a Dutch tertiary hospital. The detection of norovirus is clearly associated with the occurrence of vomiting and diarrhea. The presence of symptoms is not related to the amount of virus in feces. Sequence analysis revealed predominance of three different genogroups. In almost half of the norovirus positive events, infection was hospital acquired.

O048

Stimulation of TLR3 or TLR9 on dendritic cells and fibroblasts limits herpes simplex virus type 1 infection in an IFNβ-dependent and -independent way

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Introduction: Viruses are recognized by various pattern recognition receptors (PRRs), which are present on many different cell types. Best characterized PRRs are the Toll-like receptors (TLRs), which are abundantly present e.g. on dendritic cells (DCs). After stimulation of TLRs by viral components, type I interferons (IFNs) are produced by DCs. Type I IFNs are antiviral cytokines which can directly limit viral infection and regulate the immune response. Interestingly, synthetic ligands are now available which can stimulate these TLRs and thereby inducing the production of type I IFNs. This suggests that these ligands might be potential drugs to limit viral infections. Therefore, we used an *in vitro* model to investigate the antiviral potencies of various TLR ligands.

Methods: Mouse bone marrow cells were differentiated into plasmacytoid and conventional DCs (pDCs and cDCs). Subsequently, both DC subsets were stimulated for 24h with various TLR ligands, including lipopolysaccharide (LPS), R-848, poly(I:C) and CpG. Supernatants were then

collected and fibroblasts (L929 cells) were subsequently incubated for 18h with these conditioned supernatants. Finally, the fibroblasts were infected with herpes simplex virus type 1 (HSV-1) for 30h and cells and supernatant were collected to determine the amount of viral copies by qPCR and infectious virus by plaque assay. We also used a neutralisation assay and ELISA to determine which type I IFNs are involved in the antiviral response.

Results: The HSV-1 infection was impressively reduced when a mixed population of pDCs and cDCs was stimulated with the TLR9 ligand CpG. This effect was entirely IFNβ-mediated. Viral copy numbers were reduced by 97% and significantly less infectious virus was present. This antiviral effect was not observed in the absence of pDCs. There was also no or limited protection of fibroblasts against HSV-1 infection when exposed to supernatant of DCs stimulated with LPS or R-848. Interestingly, not only resulted the stimulation of both DC types with the TLR3 ligand poly(I:C) in a strong antiviral effect, also fibroblasts were able to significantly limit HSV-1 infection when exposed directly to poly(I:C). This effect was however only partially dependent on IFNβ.

Conclusion: These data show that in particular CpG stimulation of pDCs results in a strong IFNβ-dependent antiviral response. In addition, poly(I:C) stimulation also induced a strong antiviral response, even in the absence of DCs. Thus, CpG-stimulated pDCs and poly(I:C) are attractive candidates to limit viral infections.

O049

Improvement tuberculosis treatment; to invent new- or optimise use of old drugs?

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In order to improve the current tuberculosis (TB) therapy, several aspects in the current treatment approach of tuberculosis need to be addressed.

First, the emergence of resistance in among isolates of *Mycobacterium tuberculosis* (Mtb) against anti-TB drugs presents one of the major challenges to public health; it clearly threatens the WHO targets of TB elimination foreseen in 2050. One factor that presumably fuels the worldwide problem of emerging resistance is the evolutionary background of Mtb. Compared to other genotypes the Beijing genotype of Mtb is significantly more likely to exhibit drug resistance, a phenomenon noted in various geographical areas. Beijing Mtb may have a higher intrinsic ability to withstand treatment with anti-TB drugs, i.e. be intrinsically less susceptible to these agents. Moreover, worldwide the proportion of patients infected with a Beijing Mtb genotype is increasing. However, the mechanism(s) underlying this development in Beijing genotype strains remains unknown, and more insight could be directive in the development of new anti-TB drugs and treatment strategies within the current regimens.

Second, research should focus on the pharmacodynamics of anti-TB drugs. For example the dosage of rifampin, one of the crucial drugs currently applied in TB treatment was established after a few trials in the 1970s and 1980s. The aim of those studies was to explore the lowest rifampin and/or pyrazinamide dosage needed to add to a base regimen of daily isoniazid and ethambutol, in order to reduce the duration of therapy into a shorter (six-month) regimen. These studies, however, were not designed to determine the optimal rifampin dosage. Therefore, adjusting rifampin dosages to maximize their therapeutic effect without increasing risk and severity of side-effects remains an important goal adding in to the knowledge base of the current therapy.

O050

Pharmacokinetics and drug susceptibility testing imply limited activity of current regimens for *Mycobacterium avium* complex disease

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Background: Treatment outcome in non-tuberculous mycobacterial (NTM) lung disease is limited, with cure rates of 50-70%. One of the important factors complicating treatment of NTM disease is the discrepancy between *in vitro* drug susceptibility test (DST) results and *in vivo* response to treatment. This is partly explained by our limited knowledge of the pharmacokinetics of drugs used in NTM disease treatment, and its relation to minimum inhibitory concentrations (MIC) of patients' isolates. Within this study, we have retrospectively assessed the pharmacokinetics and MICs of the most important drugs used in treatment of *Mycobacterium avium* complex (MAC) disease.

Methods: All patients for whom pharmacokinetic analyses and drug susceptibility testing had been performed at National Jewish Health, Denver, CO, USA, during the January 2006 to June 2010 period were retrieved from the laboratory databases. Isolates were identified as MAC by high performance liquid chromatography and AccuProbe assays. DST was performed using the radiometric BacTec 460 macrodilution method, recommended by the Clinical Laboratory Standards Institute; synergy

between rifampicin and ethambutol was assessed *in vitro* by preparing four two-fold dilutions from the MICs of the individual drugs, in combination. Synergy was defined as combined MICs> 2 times lower than MICs for the individual drugs. Pharmacokinetic measurements were done using high performance liquid chromatography and gas chromatography with mass selective detection.

Results: A total of 531 pharmacokinetic analyses were performed (201 *M. avium*, 297 *M. intracellulare*, 33 MAC-X) for 481 patients (180 *M. avium*, 270 *M. intracellulare*, 31 MAC-X). The average serum concentrations for the various drugs, the median MICs and pharmacokinetic calculations are recorded in the Table. Simultaneous use of rifampicin significantly lowered serum concentrations of macrolides (30-60%) and moxifloxacin (10-15%).

Conclusions: Despite addressing synergy between rifampicin and ethambutol, pharmacokinetic measurements reveal that serum rifampicin, ethambutol and moxifloxacin concentrations attain effective levels in a minority of patients; rifampicin use exerts significant, detrimental, effects on pharmacokinetics of macrolides and moxifloxacin. These issues may partly explain the limited outcome of treatment for NTM disease. Dosing and dosing frequency should be optimized to improve outcomes. More research is needed to assess other factors involved in the discrepancies between *in vitro* drug susceptibility and *in vivo* outcome of therapy with these drugs.

Table: Average serum concentrations and pharmacokinetic calculations

DRUG	MEAN CMAX	MEAN AUC	PD TARGET	MEDIAN MIC	% ABOVE PD TARGET
Rifampicin (n = 299)	18.55± 6.75	68.42± 24.26	AUC/MIC > 271 Free AUC/MIC > 24.14	2*	6% 18%
Ethambutol (n = 421)	2.24± 1.02	10.18± 4.35	Free C_{max}/MIC > 1.23	1*	57%
Azithromycin (n = 367)	0.32± 0.23	1.47± 1.00	n.a.	n.a.	n.a.
Clarithromycin (n = 59)	2.26± 1.87	10.67± 9.53	T50%> MIC	≤4	n.a.
Moxifloxacin (n = 96)	4.25± 1.51	18.81± 6.46	C_{max}/ MIC > 10; AUC/MIC > 100	2	11%; 0%

*MIC50 and MIC90 based on MICs for rifampicin and ethambutol in combination

n.a., not applicable

O051

Minimum inhibition concentration of first and second line drugs against *Mycobacterium tuberculosis* complex isolates in relation to genetic mutations

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Because of the emergence of multidrug resistant tuberculosis (MDR-TB) accurate drug susceptibility testing (DST) method is of the utmost importance. Moreover, a more rapid, molecular approach is needed for a timely adjustment of treatment.

In total 162 clinical *M. tuberculosis* and 3 M. bovis BCG isolates were subjected to DST; 8 pansusceptible, 109 isoniazid resistant, 4 rifampicin resistant, 1 streptomycin resistant and 33 multidrug resistant (MDR) strains, including 10 controls. Detection of mutations associated with resistance to rifampicin and isoniazid was performed using the GenoType® MTBDRplus kit. The GenoType® MTBDRsl kit was used to identify mutations associated with resistance to fluoroquinolones, aminoglycosides/ cyclic peptides and ethambutol. The growth inhibition of the *M. tuberculosis* isolates during exposure to the antibiotics isoniazid, rifampicin, ethambutol was tested by using the standard BACTEC MGIT SIRE kit.

For determination of the minimum inhibitory concentration (MIC), the antibiotics amikacin, capreomycin, ethambutol, isoniazid, ofloxacin, PAS, rifampicin, streptomycin, rifabutin, and moxifloxacin were tested at different concentrations.

The GenoType® MTBDRplus was found highly reliable to screen for resistance to isoniazid and rifampicin. If a mutation in the katG gene and/or inhA gene was detected 100% of the strains were resistant to isoniazid in the MIC testing. When no mutations in one of the two genes were found, 54% of the tested strains were still resistant to isoniazid. This implies the positive predictive value was very high 100% but the negative predictive value low; 46%. For most rifampicin resistant isolates 83% a mutation was disclosed in the rpoB gene. The positive predictive value for detection of rifampicin resistance therefore amounted to 100% and the negative predictive value 95%.

The GenoType® MTBDRsl to screen for resistance against second line drugs appeared less reliable. Three out of 4 strains with a mutation in the gyrA gene (fluoroquinolones) was found resistant to ofloxacin, and moxifloxacin in MIC testing. When no mutation in the gyrA gene was observed, 2% of the strains were still resistant to moxifloxacin and for ofloxacin this was even 46%.

If no mutation in the rrs gene (aminoglycoside) was detected, 5% of the strains were resistant to amikacin, 47% to streptomycin, and 18% resistant to capreomycin in the MIC testing. If a mutation in the rrs gene was found, 67% of the strains were sensitive to amikacin, 0% sensitive to streptomycin, and 33% to capreomycin.
For the embB gene (ethambutol) only 56% of the strains with a mutation were resistant in the MIC test. When no mutations were found, 8% of the strains were resistant to ethambutol in the MIC test.
The GenoType® MTBDRplus is an useful test to screen for resistance to isoniazid and rifampicin. The GenoType® MTBDRsl in is an unreliable method to trace resistance to one of the fluoroquinolones aminoglycosides/cyclic peptides (capreomycin, kanamycin and amikacin) or ethambutol. The results of this test should be considered with great caution.

O052

Energy metabolism, diarylquinolines and pyrazinamide: insight in the molecular mechanism of drugs that may shorten tuberculosis treatment

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Introduction: Infections with *Mycobacterium tuberculosis* lead to nearly 2 million deaths per year with about 2 billion people latently infected. Multi-drug resistant strains pose a global health challenge. A key factor for drug resistance is the long duration of tuberculosis (TB) chemotherapy (> 6 months), which is mainly due to mycobacteria staying dormant within the human host. For shortening of TB chemotherapy, development of drugs that are active on the dormant metabolic state and interfere with new targets is urgently needed.
Diarylquinolines (DARQ), a new class of anti-TB drugs with lead compound TMC207, and the front-line drug pyrazinamide both show strong potency on dormant mycobacteria. Moreover, combination of these drugs displayed strong synergy in a mouse model, with the potential to significantly shorten TB treatment. A better understanding of the mechanism of action of these two drugs is strongly warranted.
Results: Previously, we demonstrated that DARQs selectively target mycobacterial ATP synthase in replicating and dormant metabolic state.[1,2,3] Respiratory ATP synthesis in mycobacteria is not only essential for growth, but also required in the physiologically dormant state.[2]
Recently, we found that DARQs effectively interact with their target at low pH values and at low proton motive forces.[4] These properties may contribute to the high activity on dormant mycobacteria. DARQs are not competitive inhibitors of proton transport, but may interfere with conformational changes of ATP synthase.[4] Furthermore, ATP synthase in fast growing as well as in slow growing mycobacteria is active in ATP synthesis, but cannot invert its function to hydrolyze ATP and establish a proton motive force.[5] This feature may constitute an important mycobacterial adaptation to life under low oxygen tensions, as found during infection in human macrophages.
We show that pyrazinoic acid, the active entity of the pro-drug pyrazinamide, interferes with respiratory ATP synthesis and decreases cellular ATP levels.[6] This drug may have multiple targets, whose relative importance needs to be assessed.
We will also present first data on synergy between these two drugs on mycobacteria grown *in vitro* and discuss possible explanations for this synergistic behavior in terms of molecular mechanism and bacterial metabolic response.
Conclusion: Respiratory ATP synthesis may be a critical weakness of (dormant) mycobacteria and drugs acting on this pathway appear to be very powerful. A better understanding of mycobacterial energy metabolism and mechanisms of drug action is needed. Moreover, insight in drug synergy, as exemplified by DARQs and pyrazinamide, may provide a blueprint for drug regimens suitable for shortening tuberculosis treatment.

References

1. Koul et al. (2007) Nat. Chem. Biol. 3, 323-4
2. Koul et al. (2008) J. Biol. Chem. 283, 25273-80
3. Haagsma et al. (2008) Antimicrob. Agents Chemother. 53, 1290-1292
4. Haagsma et al. (2011) PLoS One. 6(8):e23575.
5. Hagsma et al. (2010) FEMS Microbiol. Lett, 313, 68-74
6. Lu et al. (2011) Antimicrob. Agents Chemother. 55, 5354-5357

O053

Outer membrane proteins in mycobacteria

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Scientific interest in mycobacteria has been sparked by the medical importance of *Mycobacterium tuberculosis* and by properties that distinguish them from other microorganisms. In particular, mycobacteria produce a remarkably complex cell wall whose central component is a gigantic macromolecule formed by mycolic acids covalently attached to an arabinoga-

lactan-peptidoglycan co-polymer. The mycolic acids are long chain fatty acids which constitute the inner leaflet of a unique outer membrane of extremely low permeability.[4]

MspA was the first outer membrane protein discovered in mycobacteria.[6] It is the main porin of *Mycobacterium smegmatis* and provides the major diffusion pathway for hydrophilic solutes.[8,9]

MspA is a homo-octameric channel-forming protein.[3] The goblet-like structure of MspA has a single short (~0.5 nm) constriction consisting of two amino acids (D90, D91) with a diameter of ~1 nm.[3] MspA has more than 60 homologs in other mycobacteria and, thus, provides a compelling example that mycobacteria produce outer membrane proteins with similar functions but different structures compared to gram-negative bacteria. In addition, MspA has been used in nanotechnology applications such as DNA sequencing which profit from the higher signal-to-noise ratio of current fluctuations through the short constriction zone of MspA.[1,2]

Mycobacterium tuberculosis has no homologs of MspA and identification of other outer membrane proteins has been challenging.[5] Several proteins have been proposed as outer membrane proteins of *M. tuberculosis*, but none were verified. For example, Rv0899 (OmpATb) was proposed to be a porin of *M. tuberculosis*, but the protein does not have detectable channel activity in mycobacteria.[7] The recently solved NMR structure revealed that Rv0899 does not form a transmembrane β-barrel or any other pore-forming domain,[10] supporting the *in vivo* results. Instead, we provided evidence that Rv0899 is involved in ammonia release and acid adaptation by *M. tuberculosis*.[7]

In our search for porins of *M. tuberculosis*, we identified MtpA in a library of ampicillin resistant mutants of a genetically very similar organism, *Mycobacterium bovis* BCG. Surface accessibility experiments indicate that MtpA is an outer membrane protein. It is required for uptake of glycerol and for *in vivo* growth in macrophages. While an MtpA mutant did not grow on medium with glycerol as the sole carbon source, its growth on glucose was improved compared to the wild-type strain. Microarray analysis revealed that expression of the unrelated *mtpB* gene was significantly upregulated when the *mtpA* mutant was grown on glucose. The ability of *mtpB* to complement growth and uptake defects of an *M. smegmatis* triple porin mutant for glucose in combination with its localization in the outer membrane and its channel activity in lipid bilayer experiments confirmed its role as a porin. Further characterization of the novel porins MtpA and MtpB will reveal their roles in the physiology, pathogenicity and antibiotic resistance of *M. tuberculosis*.

References:

1. Butler TZ, Pavlenok M, Derrington IM, et al. Single-molecule DNA detection with an engineered MspA protein nanopore. Proc Natl Acad Sci U S A. 2008;105:20647-52.
2. Derrington I M, Butler TZ, Collins MD, et al. Nanopore DNA sequencing with MspA. Proc Natl Acad Sci U S A. 2010;107:16060-5.
3. Faller M, Niederweis M, Schulz GE. The structure of a mycobacterial outer-membrane channel. Science. 2004;303:1189-92.
4. Hoffmann C, Leis A, Niederweis M, et al. Disclosure of the mycobacterial outer membrane: Cryo-electron tomography and vitreous sections reveal the lipid bilayer structure. Proc. Natl. Acad. Sci. U S A. 2008;105:3963-7.
5. Niederweis M, Danilchanka O, Huff J, et al. Mycobacterial outer membranes: in search of proteins. Trends Microbiol. 2010;18:109-16.
6. Niederweis M, Ehrt S, Heinz C, et al. Cloning of the mspA gene encoding a porin from Mycobacterium smegmatis. Mol. Microbiol. 1999;33:933-45.
7. Song H, Huff J, Janik K, et al. Expression of the ompATb operon accelerates ammonia secretion and adaptation of Mycobacterium tuberculosis to acidic environments. Mol Microbiol. 2011;80:900-18.
8. Stahl C, Kubetzko S, Kaps I, et al. MspA provides the main hydrophilic pathway through the cell wall of Mycobacterium smegmatis. Mol. Microbiol. 2001;40:451-64 (Authors' correction appeared in Mol. Microbiol. 57, 1509).
9. Stephan J, Bender J, Wolschendorf F, et al. The growth rate of Mycobacterium smegmatis depends on sufficient porin-mediated influx of nutrients. Mol. Microbiol. 2005;58:714-30.
10. Teriete P, Yao Y, Kolodzik A, et al. Mycobacterium tuberculosis Rv0899 adopts a mixed alpha/beta-structure and does not form a transmembrane beta-barrel. Biochemistry 2010; 49:2768-77.

O054

Outer membrane proteins in gram-negative bacteria: structure, function and biogenesis

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The cell envelope of gram-negative bacteria is composed of two membranes separated by the periplasm containing the peptidoglycan layer. The outer membrane (OM) is an asymmetrical bilayer consisting of phospholipids in the inner and lipopolysaccharides (LPS) in the outer leaflet. The OM contains integral membrane proteins (OMPs) and peripherally associated lipoproteins, which are attached to the membrane via a lipid anchor.

The structure of OMPs is entirely different from that of other membrane proteins, which usually span the membrane via hydrophobic a-helices. OMPs are -barrels consisting of amphipathic -strands. This deviant structure is related to the biogenesis of these proteins, which have to be able to pass the inner membrane to reach their destination. Similar -barrel proteins are found in the OMs of mitochondria and chloroplasts, probably reflecting the endosymbiont origin of these eukaryotic cell organelles.

Most OMPs are involved in transport processes. Nutrients mostly pass the OM by passive diffusion via channel-forming proteins, known as porins. However, diffusion is an option only when the extracellular concentration of the nutrients is high. The uptake of dilute solutes requires energy. The concentration of iron, for example, is kept low in the vertebrate host by the presence of iron-binding proteins such as lactoferrin and transferrin. Many pathogens respond to iron limitation by the production of

iron-chelating compounds, called siderophores. They also produce receptors that bind iron-siderophore complexes. The subsequent uptake of the siderophores requires the energy of the proton-gradient across the inner membrane, which is coupled to the transport process in the OM via the TonB complex. Some pathogens don't produce siderophores, but they produce receptors that bind iron-binding proteins of the host. The acquisition of iron from these proteins is also dependent on the TonB complex. Efficient iron-acquisition mechanisms are important virulence factors and have been studied extensively in many pathogens. However, these studies have largely ignored that the availability of other metals is also limiting for bacterial growth in the host. Recent studies have revealed that several TonB-dependent receptors are not involved in iron acquisition, but in scavenging of other metals, such as zinc. Other OMPs have diverse functions, such as anchoring of the OM to the peptidoglycan, adhesion of bacteria to host cells, protein secretion and drugs extrusion. The OM contains only few enzymes, which are involved, for example, in the degradation of antimicrobial peptides or in the modification of LPS in order to evade the innate immune system. Only two OMPs have been shown to be essential for bacterial viability under laboratory conditions: LptD is needed for the transport of LPS to the bacterial cell surface and BamA is part of a complex that inserts OMPs into the OM. BamA is evolutionarily highly conserved: a homolog is also found in the mitochondrial OM, where it exerts a similar function. Because of their essentiality and their accessibility at the bacterial cell surface, LptD and BamA are interesting vaccine candidates and targets for the development of new antimicrobial compounds.

O055

Differential detergent extraction of *Mycobacterium marinum* cell envelopes reveals novel, extensively modified, outer membrane protein

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Mycobacterium tuberculosis is one of the most successful bacterial pathogens, responsible for the death of approximately 1.4 million people each year. This bacterium is characterized by lipid-rich cell envelope, which forms a thick permeability barrier and provides its high resistance to antibiotics. The cell wall comprises a polymer of peptidoglycan and arabinogalactan, covalently linked to long fatty acids, the mycolic acids, which form the inner leaflet of an atypical outer membrane, the mycolate outer membrane (MOM). Very few proteins residing in this membrane have yet been identified, despite their evident importance for nutrient uptake and secretion processes. So far, the only mycobacterial outer membrane proteins (MOMPs) described are the general porin MspA specific to *Mycobacterium smegmatis* and the channel-forming protein MctB of *M. tuberculosis*. These proteins do not have structures similar to the gram-negative outer membrane proteins, as they reside in an unique lipid bilayer. Due to their location on the outer layer of the bacteria, these proteins represent attractive drug targets, but their discovery is complicated by the difficulties in obtaining clean fractions of mycobacterial inner- and outer membranes.
To identify novel MOMPs, we have developed a reproducible method for differential fractionation of mycobacterial proteins of the inner- and outer membrane. During these studies we discovered that we can specifically obtain proteins from the outer layer of *Mycobacterium marinum* cell envelopes using differential solubilisation with specific detergents. These extracted fractions were analyzed by mass spectrometry (LC-MS) to determine which proteins are located in the MOM. This showed that marker protein for IM and MOM were found in their expected fractions and revealed a few interesting candidate MOMPs. A number of the candidate MOMPs were further analyzed for their expression and localization in the cell envelope. One of these identified MOMPs was purified and demonstrated to form a large multimeric complex. In lipid bilayer experiments this protein clearly showed channel activity, indicating this protein is a novel porin. The most surprising feature of this protein was the presence of a long threonine-rich domain with extensive modifications, resulting in a 40% increase of the total mass of the protein. Currently, we are analysing the exact nature of these modifications, which probably include glycosylation. In conclusion, we have identified a novel mycobacterial outer membrane protein with very unusual characteristics.

O056

Omp32 of *Helicobacter pylori* is involved in nickel and cobalt transport

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Introduction: *Helicobacter pylo*ri is able to colonise the acidic environment of the stomach where it causes gastritis and infection can result in peptic ulcers and cancer. Nickel plays an essential role in the pathogenesis of this organism since both nickel co-factored enzymes, hydrogenase and urease, are essential for colonisation. At high concentrations nickel is toxic; therefore the intracellular nickel concentration is strictly maintained by the nickel-responsive regulator

NikR, which represses transcription of nickel acquisition genes. The presence of a NikR binding site in the promoter region of a gene is therefore suggestive of its involvement in nickel homeostasis. Previously, the nickel-regulated TonB2-dependent receptor FrpB4 (hp1512), was demonstrated to be involved in transport of nickel across the outer membrane. However the exact mechanism of nickel transport is not completely understood. Here we report the characterisation of a putative porin, Omp32 (Hp1501), which contains a putative NikR binding site overlapping its transcription start site.

Methods: The intracellular metal content of the wild type and isogenic *omp32* and nikR mutants was measured using induction-coupled mass spectrometry. Metal toxicity was assessed using broth cultures with increasing concentrations of cobalt and nickel. Acid shock experiments in Brucella broth at pH 5.5 were performed to induce urease expression and increase the intracellular demand for nickel; subsequently nickel-dependent urease activation was measured at different time intervals.

Results: Inactivation of *omp32* resulted in strongly reduced nickel and cobalt accumulation and in reduced urease activity. To investigate the role of Omp32 in TonB2-mediated nickel transport, *tonB2* was mutated in the *omp32* mutant, this did not further reduce the urease activity. In contrast with the *omp32* mutant the *nikR* mutant had an increased nickel and cobalt accumulation and increased urease activity. Surprisingly, the iron content was reduced in both *omp32* and *nikR* mutants. Toxicity assays revealed that an *omp32* mutant was more resistant to nickel and cobalt than the wild type. An acid shock activated the urease enzyme in intact wild type cells but only in the presence of extracellular nickel; this urease activation was absent in both the *tonB2* and the *omp32* mutant.

Conclusion: Our results suggest that Omp32 is part of the previously described TonB2-FrpB4 dependent nickel transport system. Surprisingly, this transport system is not specific for nickel but also transports cobalt, the cofactor of *H. pylori* arginase, which metabolises arginine to ornithine and urea. The essential role of urease and hydrogenase in colonisation and their nickel dependency makes the TonB2-FrpB4-Omp32 system a potential target for therapeutic agents to combat *H. pylori* infection.

O057

Detection of genes essential for growth of respiratory pathogens

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Introduction: Respiratory tract infections are a leading cause of global mortality and morbidity. It has been estimated by the WHO that annually 4-5 million people die of pneumonia. Infections that rarely lead to death include sinusitis and otitis media, and are the second most common disease of childhood after upper respiratory infection in developed countries. Infection by and growth of a respiratory pathogen is a complex process dependent on a number of essential pathways, of which members could form ideal candidate targets for drug design and/or vaccine development

Methods: To identify microbial genes essential for growth of respiratory pathogens, we have used an insertion knockout strategy and developed a bioinformatics tool that allows rapid identification of disrupted genes. This method employs the next generation sequencing method Tn-Seq to generate footprints of bacterial transposon mutant libraries. Genes that are not detected in the knockout library are likely essential for growth if a sufficiently large knockout library is used. To identify shared essential pathways in bacterial species we have used statistical analysis, pathway analysis and functional category enrichment methods to determine these ideal candidates for drug design and vaccine development.

Results: In *Streptococcus pneumoniae* as well as in the two other bacterial respiratory tract pathogens *Moraxella catarrhalis* and *Haemophilus influenzae* we observed that roughly 15% of all genes is essential, similar to what has been found in classical knockout studies. Most of these genes encode functions involved in transcription, translation or replication, however several genes encoding hypothetical proteins are also included. The products of these genes possibly play a role in essential processes in the bacterial cell and might form interesting novel candidate targets for drug design and/or vaccine development

Conclusions: High throughput screening of essential genes is feasible using Tn-Seq. To this end, a large knockout library is preferred to decrease the number of false positives. Importantly, genes encoding for orthologous proteins in all three species have been found to be essential. These include hypothetical proteins, which are likely to play important roles in critical processes in the cell.

O058

Unraveling the small regulatory RNA network of *Bacillus subtilis*

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In the post genome era it has become clear that there is a large portion of genomes that, although not protein coding, is transcribed. These ncRNAs (non coding RNAs) include 5' and 3' UTRs (untranslated regions), intergenic regions, and completely independently transcribed segments. All of these classes contain antisense RNAs. It is now a challenge not only to determine which areas of a genome are non-coding, but also to determine what their function is within the cell.

sRNAs (small regulatory RNA's) regulate their target mRNAs via specific complementary base pairing at the post transcriptional level, leading to stabilization or degradation of the RNA complex and can thereby act as distinct, specific regulatory hubs. To date most sRNA functions are known in Gram negative bacteria where sRNAs are involved in virtually all cellular processes studied, including virulence. Large questions remain however about the importance and molecular mechanisms of these elements in Gram positive bacteria.

An extensive study was performed on the versatile bacterium *Bacillus subtilis*, where it was grown in 104 different experimental conditions, and the respective RNA hybridized to high resolution tiling arrays (submitted). From this, we now have a dataset of 1500 new non coding RNAs of *B. subtilis*, many of which are condition specific. This rich dataset is currently being exploited to predict the function and regulatory targets of the detected likely sRNAs (subset of independent segments, n = 43) using a multifaceted approach.

This consists of among other things; documenting specific sRNA expression conditions, conservation analysis, secondary structure predictions of all ncRNAs, extensive sRNA target predictions, enrichment of functional categories in the target predictions and studying expression correlations between sRNA and putative targets under relevant environmental conditions. Since sRNA target predictions are intrinsically noisy, our approach is focused on filtering out as many false positive predictions as possible. At this stage our analysis pipeline is especially useful in prediction of sRNA – target interactions involving regulation of RNA stability and / or decay.

Our predictions have led to uncovering some intriguing phenotypes involving stress tolerance and protein secretion. In addition, we have found that putative sRNAs (independent segments) have a higher secondary structure and are more conserved through evolution than the other ncRNA classes. We are currently using several experimental approaches to verify the sRNA target predictions. These include transcriptomics, advanced proteomics and target verifications with reporter genes. We expect this work to ultimately provide a Systems Biology view of sRNA mediated gene regulation in *B. subtilis*.

O059

From transcriptional landscapes to prediction of stress induced robustness using biomarkers

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Introduction: Microorganisms face changing environmental conditions in their natural habitats, in foods and in the gastro-intestinal tract. They have evolved sophisticated stress adaptation mechanisms to be prepared for challenges even before they arise. The stress adaptive response is a crucial survival strategy for a wide spectrum of microorganisms, and can result in increased robustness. Scavengers of reactive oxygen species (ROS) have a crucial role in controlling and adapting to oxidative stresses which microorganisms encounter during disinfection treatments and as part of the host defence response. The significant role of ROS scavengers could point to their potential role as biomarker for stress induced robustness. Prediction of stress induced enhanced robustness using biomarkers will allow to better understand stress adaptation mechanisms and to control stress adaptive traits.

Methods: We designed a framework for identifying molecular biomarkers for mild stress induced microbial robustness towards lethal stresses. Several candidate-biomarkers were selected by comparing the genome-wide transcriptome profiles of our mesophilic model organism *Bacillus cereus* upon exposure to four mild stress conditions (heat, acid, salt and oxidative stress). Expression of scavengers of reactive oxygen species including catalases was demonstrated in the transcriptional adaptation profiles to all four stresses. The predictive quality of catalase activity as biomarker for stress induced robustness was evaluated for *Bacillus cereus* using the designed framework. Induction of catalase activity upon mild oxidative stress treatment seemed to be a good predictor for mild stress induced robustness. The predictive quality of catalase activity was therefore also evaluated for the psychrotolerant species *Bacillus weihenstephanensis*. Catalase activity was quantitatively determined in cells cultured at 30°C and 7°C and exposed to mild oxidative stress for various time intervals. Both unstressed and mildly stressed cells were also exposed to lethal stress conditions (heat, acid and oxidative stress) to quantify the robustness advantage provided by mild oxidative stress pretreatment. To evaluate whether catalase activity could predict the robustness level of mild stress treated cells, the induction upon mild stress treatment was correlated to mild stress induced robustness towards lethal stress. The correlation significance was evaluated using the Pearson correlation coefficient.

Results: Mild oxidative stress treatment provided cross-protection towards lethal heat, acid and oxidative stress. Increased catalase activity was demonstrated upon mild oxidative stress treatment and these induction levels were

significantly correlated to induced enhanced robustness towards lethal stresses. This highlighted the significant role of catalase activity in mild oxidative stress adaptation. Additionally, this underlined the good predictive quality of catalase activity as biomarker for mild stress induced robustness towards lethal stresses.

Conclusion: Scavenger of reactive oxygen species have an important role in stress adaptation. We demonstrated that catalase activity could serve as biomarker for stress adaptive behaviour in the mesophilic species *Bacillus cereus*, and also in the psychrotolerant species *Bacillus weihenstephanensis*. Our study provides a systematic, quantitative approach to search for biomarkers for adaptive behaviour and to statistically evaluate their predictive potential to select biomarkers with high predictive quality for several species that can serve to early detect and predict adaptive traits.

O060

Characterisation of the biodiversity of spoilage *Lactobacilli*

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Members of the genus *Lactobacillus* are associated with the spoilage of specific foods by production of gas or excessive acid. In practice a cocktail of isolates that previously caused spoilage of products is used to test the stability of new food (product) designs. The design of stable and yet minimally processed and mild foods requires representative isolates as well as knowledge of the microbial targets for preservation. Thereby we are performing a thorough genotypic and phenotypic characterisation of the multiple *Lactobacillus* species and strains previously associated with spoilage of food products.

Genetic analysis commenced with sequencing the genome of multiple strains of four key *Lactobacillus* species involved in spoilage. These genomes were then compared *in silico* to the genomes of reference *Lactobacillus* strains. Phenotypic analysis was performed based on a previously defined set of growth tests (mainly sugar metabolism) for the differentiation of *Lactobacillus plantarum* strains. These assays proved to be valuable in the differentiation of 120 strains from different *Lactobacillus* species. A second set of phenotypic assays was designed to assess the ability to grow under various preservation conditions, such as low pH, in the presence of lactic acid or acetic acid, at low temperature and water activity, or in the presence of high salt levels. Strikingly, the variation in phenotypic properties between strains was larger than that between the species tested. This suggests that spoilage isolates are not specific strains that are able to grow in a preserved food but rather incidentally entered a food product. Our further research will involve integrating the phenotype and genotype datasets to identify marker genes for spoilage behaviour.

O061

Protein complexes involved in the electron transport chain of anammox bacteria

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Anaerobic ammonium oxidizing (anammox) bacteria combine ammonia with nitrite to dinitrogen gas with nitric oxide and hydrazine as intermediates. Oxidation of the latter yields low-redox-potential electrons, which can be used for CO2 fixation via the reductive acetyl-CoA pathway. We hypothesize that these are replenished through the oxidation of nitrite to nitrate by a nitrite oxidizing system (NAR). As nitrite is a relatively poor reductant, the electrons have to be energized to enter the bc1-complex or to feed a quinone pool, which implies reverse electron transport.

The gene cluster that contains the catalytic subunits of nitrite oxidizing system (narGH) covers almost the full natural repertoire of electron carriers. This includes genes encoding six putative heme-containing proteins and two putative blue-copper proteins and a putative anchor to the membrane showing homology to a cytochrome bd oxidase subunit.

Furthermore, the genome of the anammox bacterium Candidatus Kuenenia stuttgartiensis shows a high redundancy of respiratory genes, suggesting an intricate cellular electron transport system. Interestingly, the three operons encoding for the bc1 complexes, complex III in the respiratory chain, all differ in their subunit composition from the canonical bc1 complexes in other microorganisms. One operon consists only of a heme b /c fusion protein and the Rieske protein. The other two operons encode for multi heme c containing genes, NAD(P) oxidoreductase subunits and, intriguingly one of them contains a hydroxylamine oxidoreductase subunit.

The combination of these subunits strongly suggests that electrons derived from different oxidation reactions could be wired to different electron acceptors, once entering the bc1 complexes.

The whole protein complement of K. stuttgartiensis membranes was determined with protein correlation profiling using LC-MS/MS data from consecutive Blue Native (BN) gel slices. The detection of different complexes

was coupled to in-gel activities of the respiratory complexes in BN gels. Further, the catalytic subunit of the nitrite oxidizing system of K. stuttgartiensis was purified.

O062

A multi-platform flow device for microbial cultivation and microscopic analysis

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Novel microbial cultivation platforms are of increasing interest to researchers in both academia and industry. The development of materials with specialized chemical and geometric properties and has opened up new possibilities in the study of previously unculturable micro-organisms, and has facilitated the design of elegant, high-throughput experimental set-ups. Within the context of the international genetically engineered machine (iGEM) competition, we set out to design, manufacture and implement a flow device that can accommodate multiple growth platforms, i.e., a silicon nitride based microsieve and a porous aluminium oxide based microdish. It provides control over culturing conditions similar to a chemostat, while allowing organisms to be observed microscopically in real-time. The device was designed to be affordable, reusable, and above all, versatile. To test its functionality and general utility, we performed multiple experiments with *Escherichia coli* cells harboring a complex, synthetic gene circuit, and were able to quantitatively study the emerging expression dynamics in real time via fluorescence microscopy. A plasmid encoding a synchronized oscillating gene circuit was constructed from standard biological parts conforming to the BioBrick assembly standard. This circuit was made up of delayed positive and negative regulatory feedback loops consisting of quorum sensing and quenching enzymes and transcription factors from Vibrio fischerii and Bacillus thuringiensis. We were able to demonstrate oscillatory protein expression of green fluorescent protein synchronized across *E. coli* microcolonies in porous aluminium oxide microdishes over a period of approximately 6 hours. Our results are consistent with previous investigations of synchronized oscillators in *E. coli* performed on microfluidic chips. The conditions necessary to observe synchronized oscillations were not achievable using conventional 96-well fluorescence spectroscopy plates. We conclude that our platform provides a unique and useful approach to studying microbial gene expression. Furthermore, we demonstrated that the device provides a unique environment for the cultivation of nematodes, suggesting that the device could also prove useful in microscopy studies of multicellular microorganisms.

O063

A case of New Delhi metallo-beta-lactamase 1 (NDM-1) in the Netherlands with secondary transmission

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Introduction: Acquired carbapenemases such as NDM-1 are emerging resistance determinants in gram-negative bacteria, leaving few or no therapeutic options. Here we describe a NDM-1 case in the Netherlands, imported from the Balkan area, and a secondary case in the same hospital.
Methods: The study was performed as part of a retrospective analysis on a collection of 485 *Enterobacteriaceae* isolates, from patients in the east of the Netherlands. Antimicrobial susceptibility testing and species identification were performed by the VITEC 2. Cabapenems MIC values were determined by the E-test. Phenotypic ESBL confirmation was performed by a combination disc diffusion. For phenotypic confirmation of carbapenemase production, the modified Hodge test (MHT) and two inhibitor-based tests: ertapenem-boronic acid and imipenem-EDTA, were used. A microarray was used for genotypic characterisation of ESBL's and carbapenemases. Characterisation of NDM-gene and plasmid analysis, were done using PCR, sequencing and cloning. Genetic relatedness is tested by Amplified Fragment Length Polymorphism (AFLP). For genotyping the Multilocus sequence typing (MLST) was used.
Results: Two ESBL producing *K. pneumoniae* strains from two patients were selected for further analysis by their elevated MIC's to meropenem. One patient was transferred from a hospital in Belgrado, Bosnia, to hospital in the east of the Netherlands on the 27th of August 2008 and directly placed in a separate room in isolation because of MRSA carriage. The second patient, with no travel history outside the Netherlands, was admitted to the same hospital between the 10th of Oktober and the 7th of November 2008. Antimicrobial susceptibility testings ans phenotypic tests showed elevated MIC's for carbapenems and other agents, and conformed the presence of ESBL and carbapenemase production. Molecular gene analysis revealed NDM-1, CTX-M-15, SHV-12,TEM-1, and OXA-1. Molecular plasmid analysis revealed the presence of a 70 kb, Inc II plasmid containing the bla NDM-1, and a 140 kb plasmid in both isolates. AFLP showed that both strains were identical. MLST analysis showed that both isolates belong to ST15.
Conclusion: This first encountered NDM-1 producing *K. pneumoniae* in the Netherlands underlines further that Balkan states constitute a secondary reservoir for NDM-1 producers. While cross transmission in Europe of NDM-1

producers has sporadically been reported, the index case was associated with a secondary case, although the route of transmission remains unknown.

O064

Prevalence of rectal carriage of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in hospitalised patients: 2010 and 2011

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Introduction: The prevalence of extended spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E) is rapidly increasing worldwide in both hospital and community settings. This study presents the prevalence of rectal carriage of ESBL-E in hospitalised patients in 2010 and 2011.

Methods: Two prevalence surveys were performed in the autumn of 2010 and 2011 in a Dutch teaching hospital. Rectal swabs were taken from all patients hospitalised on the day of the survey, including day-care patients. Swabs were placed in a selective tryptic soy broth, containing cefotaxime (0.25 mg/L) and vancomycin (8 mg/L) (TSB-VC). After 18-24 hours incubation (35-37°C) the TSB-VC was subcultured on a selective ESBL screening agarplate (EbSA,Cepheid) that was read after another 18-24 hours of incubation (35-37°C). Species identification and susceptibility testing was performed for all isolates that grew on the EbSA agar plate using VITEK2 (bioMérieux). For isolates with a MIC for ceftazidime and/or cefotaxime > 1 mg/L the presence of ESBL was phenotypically confirmed using the combination disk diffusion method for cefotaxime, ceftazidime and cefepime, both with and without clavulanic acid (Rosco). Test results were considered positive if the inhibition zone around the disk was increased ≥ 5 mm for the combination with clavulanic acid. Genotypic confirmation of the presence of ESBL genes was performed for all phenotypic ESBL positive isolates with the Check-MDR CT103 micro array (Check-Points).

Results: Rectal swabs were obtained from 559 of 668 (84%) and 572 of 638 (90%) hospitalised patients in 2010 and 2011, respectively. The prevalence of ESBL-E carriage was 4.1% (23/559) in 2010, and 4.7% (27/572) in 2011 (p = 0.664).*Escherichia coli* was the predominant ESBL-positive species identified, both in 2010 (96%) and 2011 (83%). Where in 2010 all ESBL-E belonged to the group I *Enterobacteriaceae*, in 2011 17% (4/29) of the ESBL-E belonged to group II (p = 0.064).

For both surveys, age, sex, stay on the ICU and length of hospital stay were not statistically significantly associated with ESBL-E carriage.

Conclusion: The overall prevalence of ESBL-E rectal carriage was comparable for the 2010 and 2011 surveys (4.1% and 4.7%, respectively). However, the relative contribution of ESBL-E of group II increased substantially from 0% to 17%. Although not statistically significant, this finding indicates that the variability in ESBL-E positive species may be increasing.

O065

Optimizing the Dutch carbapenemase detection guideline for OXA-48 producing *E. coli* associated with a large outbreak

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Introduction: The Dutch guideline for detection of carbapenemases in *Enterobacteriaceae* recommends screening for carbapenemases with a meropenem screen breakpoint of ≥ 0.5 mg/L, and for some species also with imipenem ≥ 2mg/L. Ertapenem was not advised because of low specificity. Aim of this study was to determine the test characteristics of these guideline recommendations as used for evaluation of OXA-48 producing *Enterobacteriaceae* associated with a major outbreak in the Netherlands.

Methods: Vitek susceptibility profiles and meropenem and ertapenem Etest results of genetically confirmed OXA-48 producing isolates from a large outbreak were available. Test characteristics of screening with different carbapenems were also determined for an international panel of 234 non-repeat *Enterobacteriaceae* (95 *E. coli*, 72 *K. pneumoniae*, 48 *Enterobacter* spp., 8 *K. oxytoca*, 6 *P. mirabilis*, 3 *S. marcesens*, 1 *P. stuartii*, 1 *C. freundii*) including 74 isolates producing the following carbapenemases: 36 KPC-2/3, 4 KPC plus VIM, 4 NDM-1, 6 GIM, 20 VIM, 4 OXA-48. PCR and sequencing of beta-lactamases was used as reference test. Carbapenem MICs in this panel were determined using broth microdiltuion and Etest.

Results: 431 OXA-48 producing outbreak *Enterobacteriaceae* strains with Vitek-2 MICs from 92 patients were available; 249 *K. pneumoniae*, 144 *E. coli* and 38 other *Enterobacteriaceae*. Using the meropenem ≥ 0.5 mg/L and/or imipenem ≥ 2mg/L screen criteria of the Dutch guideline, 106 (25%) of the OXA-48 producing isolates were screen negative (i.e. screen sensitivity was 75%). Per species, screen sensitivity was 93% for *K. pneumoniae*, 41% for *E. coli* and 90% for other Enterobacteriacea. Of

the 92 patients, 9 patients (10%) exclusively had screen negative isolates. Ertapenem Etest results were available of 112 isolates of 58 patients. On the isolate level, sensitivity of screening with ertapenem ≥ 0.5 mg/L, ≥ 0.25 mg/L, ≥ 0.125 mg/L was 95%, 98%, and 99%, respectively, and detected all patients with all three breakpoints.
In the international panel of *Enterobacteriaceae* isolates, using the carbapenemase screening breakpoints of the guideline had sensitivity of 100% and specificity of 80%. Screening with ertapenem ≥ 0.5 mg/L had sensitivity of 91% and specificity of 67%, although specificity differed significantly per species (93% in *E. coli*, 44% in *K. pneumoniae*, and 21% in *Enterobacter* spp).
Conclusion: Using the meropenem and imipenem screen breakpoints of the Dutch carbapenemase detection guideline would not detect 25% of OXA-48 producing outbreak strains and 10% of the patients would not be identified. Particularly, OXA-48 producing *E. coli* would not be detected. To detect OXA-48 producing *E. coli*, ertapenem with a screening breakpoint of ≥ 0.5 mg/L could be used for *E. coli* besides meropenem and imipenem.

O066

Surveillance of carbapenemase producing *Enterobacteriaceae* in the Netherlands

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Introduction: Carbapenemase producing *Enterobacteriaceae* are an important threat to health care. Besides resistance to the class of beta-lactam antibiotics, associated resistance genes to other classes of antibiotics leave very little possibilities for antibiotic therapy for infections with these strains. In several countries, outbreaks in hospitals and rapid dissemination through the health care system have been observed; a reason to start surveillance in the Netherlands.
Methods: Following the draft national diagnostic guideline. medical microbiological laboratories in the country were advised to submit all *Enterobacteriaceae* with an MIC for meropenem > 0.25 for phenotypical and genotypical confirmation for carbapenemases. Carbapenemase genes tested by PCR were: KPC, NDM, VIM, GIM, SIM, IMP, SPM, and OXA-48. Meropenem MIC was determined with Etest. Further strain- and epidemiological information was collected.
Results: A large outbreak in one hospital with an OXA-48 positive *Klebsiella pneumoniae* was detected. Data from this hospital is excluded from the description of the surveillance results.
For 2011, up to December 21st, 132 different isolates from 129 patients were submitted by 40 hospitals. Seventy one (54%) had a meropenem MIC> 0.25. Thirty different CPE were detected from 27 patients. Three times, a patient harboured two different bacterial species (*Escherichia coli* and *K. pneumoniae*) with the same carbapenemase (OXA-48, resp. NDM).
Carbapenemases found were OXA-48 (19x), NDM (5x), KPC (4x) and VIM (2x). Species involved were *K. pneumoniae* (24x), *E. coli* (3x), *Enterobacter cloacae* (2x) and Citrobacter freundii (1x). Meropenem MICs ranged from 0.38 mg/L (one OXA-48) to > 32 mg/L (11x). MICs were within sensitive range (≤ 2 mg/L) in 11 and resistant (> 8 mg/L) in 14 isolates. Seven isolates had an MIC > 8 mg/L without a detectable carbapenemase gene.
Risk factors reported for 17 patients were admission to a foreign hospital in 8 cases (Morocco 4x, Turkey, Pakistan, Libya) or the Dutch outbreak hospital in 5 cases.
Conclusion: Besides the large OXA-48 outbreak, no other CPE outbreaks were detected in 2011 through the surveillance and a limited number of CPE were seen in the Netherlands. The surveillance is on voluntary base and may therefore not have complete national coverage yet. As far as information available, the far majority of cases could either be related to the Dutch outbreak hospital or to admissions in a foreign hospital. Klebsiella pneumonia was the predominant species (80%) and OXA-48 the most frequently encountered (63%) carbapenemase.

O067

The impact of clinical breakpoint changes on surveillance of antimicrobial resistance in *Enterobacteriaceae* causing bacteraemia

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Introduction: Surveillance is an essential tool to monitor antimicrobial resistance rates. However, changes in clinical breakpoints might influence the outcome and implications of this surveillance. Dutch laboratories are currently changing from mostly CLSI (Clinical Laboratory and Standards Institute) to EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines. To evaluate the impact of these changes on surveillance, we compared trends in resistance among *Enterobacteriaceae* interpreted according to the EUCAST and CLSI guidelines, and of the antimicrobial susceptibility test (AST) results as reported by Dutch laboratories participating in the Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR).

Methods: ISIS-AR has connected 27 Dutch medical microbiology laboratories to a central database where AST results, including minimal inhibitory concentration (MIC) values and disk zone diameters (if available), of routinely cultured bacterial species are uploaded on a monthly basis. We included all blood specimens with *Enterobacteriaceae* and their susceptibility results to amoxicillin (AMO), amoxicillin/clavulanate (AMC), piperacillin/tazobactam (PTZ), cefuroxim (CFX), ceftazidime (CAZ), cefotaxim (CTX), imipenem (IPM), gentamicin (GEN), ciprofloxacin (CIP) and co-trimoxazole (SXT) from January 2008 until July 2011 (n = 15548). MIC values of Etests or automated systems were used to compare AST results using EUCAST 2011 and CLSI 2011 guidelines to AST results reported by the laboratories. If MIC values were unavailable, disk diffusion zone diameters were used. Time trends were analyzed by generalized linear models with a negative binomial distribution.

Results: EUCAST recommends lower MIC breakpoints defining resistance for AMO, AMC, PTZ, CFX, CAZ, GEN and CIP than CLSI, resulting in higher resistance rates when using EUCAST guidelines compared to CLSI guidelines. When time trends were evaluated, resistance to CFX, PTZ and GEN increased significantly since 2008, regardless of the AST method (i.e., guideline or laboratory result) used. However, resistance to AMC and CAZ increased significantly when AST results reported by the laboratories were used, while resistance to these two agents remained stable when using EUCAST or CLSI guidelines. Resistance to IPM increased only when applying EUCAST guidelines.

Conclusions:

1. Resistance rates to certain antimicrobial agents are increasing. However, the implementation of EUCAST guidelines is likely to explain a significant part of these increases in resistance.
2. Reported resistance rates are expected to further increase with the more widespread implementation of EUCAST guidelines among laboratories in the Netherlands.
3. This study illustrates the importance of collecting MIC values and not only the AST results reported by laboratories to effectively monitor resistance trends in surveillance programs.

O068

Cefotaxime resistant *Enterobacteriaceae* in fecal samples of dogs and cats

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Introduction: Extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* with resistance to extended spectrum cephalosporins, like cefotaxime, have been isolated from different reservoirs. The epidemiology of ESBLs, however, is poorly understood as data are lacking for the presence in most reservoirs and routes of transmission. The use of 3rd and 4th generation cephalosporines has been suggested as the driving force for selection of ESBLs. In companion animals, a 3rd generation, long acting cephalosporin (cefovecin) has been approved in Europe. We reported previously the isolation of ESBL and AmpC producing isolates from clinical samples of companion animals (NVVM spring meeting 2011). Intestinal carriage of ESBLs in companion animals has not been studied in the Netherlands. This knowledge is important to identify the potential source of clinical isolates and to determine the role of companion animals in the transmission to humans. The aim of this pilot study was to determine the presence of cefotaxime resistant *Enterobacteriaceae* in the gut of healthy dogs, and dogs and cats with diarrhea.

Methods: Faecal samples from healthy dogs (n = 17) were collected from different parts of the Netherlands. Samples from dogs (n = 11) and cats (n = 6) with diarrhea were obtained from the Veterinary Microbiological Diagnostic Center (VMDC) of the Faculty of Veterinary Medicine. Each sample was inoculated onto MacConkey agar supplemented with 1 mg/L cefotaxime (MacConkey+) and LB-medium supplemented with 1 mg/L cefotaxime. After overnight incubation, this enrichment was plated onto MacConkey+. From the enrichment 1 suspected colony and from the direct plating, the species of 5 suspected colonies were biochemically identified.

Results: Among the healthy dogs, 11/17 samples were positive after direct plating with high numbers of colonies. Eight samples harbored *E. coli* (confirmed for 5 colonies per sample). One sample showed growth of *Pseudomonas* and 2 samples showed growth of other non-*Enterobacteriaceae* species. Enrichment showed the same results.

Of the dogs with diarrhea, 6/11 samples were positive after direct plating with high numbers of colonies. Five showed growth of *E. coli* (confirmed for 5 colonies per sample) and one sample showed growth of a non-*Enterobacteriaceae* species. Enrichment showed 7/11 samples positive, all *E. coli*.

Among the cats with diarrhea, 4/6 samples were positive after plating with 3 samples with *E. coli* and 1 with both *E. coli* and *Proteus* spp. After enrichment, only *E. coli* was isolated from these 4 positive samples.

ESBL/AmpC-production of the isolates is currently confirmed by combination disk diffusion tests. As the study is extended up to 20 animals for each animal species-clinical status, data on 80 animals will be presented at the meeting.

Conclusion: Preliminary data of this pilot study already shows a high prevalence of cefotaxime resistant *Enterobacteriaceae* (mainly *E. coli*) in dogs and cats with diarrhea, and in healthy dogs. The fact that almost all samples were positive after direct plating suggests the presence of large amounts of ESBL producing *Enterobacteriaceae* in the gut of companion animals. Due to close contact between companion animals and humans there is a potential risk for transfer of ESBLs.

O069

Towards predicting the antigenic evolution of influenza A virus

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Human influenza viruses are classic examples of antigenically variable pathogens that have a seemingly endless capacity to evade the host's immune response. The viral hemagglutinin (HA) and neuraminidase (NA) proteins are the main targets of our antibody response to combat infections. HA and NA change continuously to escape from humoral immunity, a process known as antigenic drift. The WHO coordinates a global influenza surveillance network that routinely characterizes the antigenic properties of circulating strains by the hemagglutination inhibition (HI) assay to select new seed viruses for vaccine updates. To facilitate a quantitative interpretation and easy visualization of HI data, a new computational technique called 'antigenic cartography' was developed (1). Since its development, antigenic cartography has become a core component of the WHO surveillance activities. Antigenic cartography has greatly facilitated the visualization of trends in the global circulation of influenza viruses (2).
Until recently, antigenic variation was not considered a serious issue for avian influenza. However, because of the diversification of the Asian H5N1 lineage since 1996 into multiple genetic clades and subclades (3), and because of the long-term use of poultry vaccines against H5N1 in some parts of the world, this issue needed to be readdressed. To this end, the antigenic properties of panels of avian H5N1 viruses were characterized by HI assay using mammalian or avian antisera, and analyzed using antigenic cartography methods. These analyses revealed considerable antigenic differences between H5N1 viruses and H5 viruses used in poultry vaccines. Moreover, considerable antigenic variation was also observed within the H5N1 virus lineage.
The molecular basis of antigenic drift was determined for HA of human influenza A/H3N2 virus and avian influenza A/H5N1 viruses. In contrast to the current dogma, only very few amino acid substitutions were responsible for major antigenic changes within the H3N2 and H5N1 virus subtypes. Amino acid substitutions with large antigenic impact occurred exclusively at positions in HA that form an antigenic ridge adjacent to the receptor-binding site. These data indicate that very few key positions in HA shape the antigenic evolution of influenza A/H3N2 virus (4,5). Consequently, the number of potential drift variants is limited and the antigenic evolution of influenza A virus is substantially more predictable than previously thought, which may aid in vaccine strain selection and the production of seasonal and (pre-) pandemic vaccine candidates.

References:

1. Smith DJ, et al. Mapping the antigenic and genetic evolution of influenza virus. Science. 2004;305:71-6.
2. Russell CA, et al. The global circulation of seasonal influenza A (H3N2) viruses. Science. 2008;320:340-6.
3. Toward a unified nomenclature system for highly pathogenic avian influenza virus (H5N1). WHO/OIE/FAO H5N1 Evolution Working Group. Emerg. Infect. Dis. 2008;14:e1.
4. Koel BF, et al. Seven positions near the H3 receptor binding site dictate 35 years of influenza virus antigenic evolution. Submitted.
5. Koel BF, et al. Mapping the antigenic determinants of influenza A/H5N1 virus. In preparation.

O070

Changes in the composition of the pneumococcal population in the Netherlands after the implementation of the 7-valent pneumococcal vaccine

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Introduction: In 2006, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in the national immunization program in the Netherlands. Due to selective pressure induced by the vaccine, the implementation of pneumococcal vaccination may lead to alterations in the pneumococcal population. The aim of this study was to investigate the possible changes in the composition of the pneumococcal population before and after the implementation of PCV7.
Methods: To monitor such changes, pneumococcal isolates causing invasive pneumococcal disease (IPD) before (2004-2005, n = 1154) and after (2008-2009, n = 1190) the implementation of PCV7 in 2006 in the national immunization program of the Netherlands were characterized by molecular typing using multiple-locus variable number tandem repeat analysis (MLVA).

Results: After the implementation of PCV7, an impressive reduction of cases was observed by vaccine serotypes in children < 5 years of age. In the age group of patients ≥ 5 years of age, the overall IPD incidence remained constant, but the IPD incidence due to vaccine serotypes declined in this age cohort as well, indicating herd immunity. In general the composition of the pneumococcal population remained similar after the introduction of PCV7, although shifts in the genetic background of the isolates belonging to serotypes 1 and 22F were observed during the period from 2001 through 2009. Both before and after introduction of the vaccine several possible capsular switch events were noticed. We found 4 isolates from the pre-vaccination period in which the serotype 19F capsular locus had been horizontally transferred to a different genetic background. Remarkably, none of the 5 post-vaccination isolates in which we observed possible capsule switch belonged to the 19F serotype, possibly due to vaccine induced pressure. In the post-vaccine implementation period we found no evidence for capsular switch of a vaccine serotype to a non-vaccine serotype, indicating that capsular switch is not the main driving force for replacement.

Conclusion: (1) The selective pressure of the vaccine does not seem to influence the genetic background of the pneumococcal population yet. (2) Fluctuations in the genetic background of serotypes 1 and 22F isolates were observed regardless of the implementation of PCV7. (3) Capsular switch is not the main driving force for serotype replacement.

O071

Mucosal immunization protects mice against influenza virus-induced pneumococcal otitis media

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Streptococcus pneumoniae (the pneumococcus) is a human pathogen that transiently colonizes the mucosal surfaces of the upper respiratory tract (URT). Children represent the major reservoir for infection and can have carriage rates of over 80%. After the first few years of age, pneumococcal carriage rates decline to below 10%. In most cases, *S. pneumoniae* colonization is transient and asymptomatic. However, an antecedent or concurrent respiratory virus infection can allow *S. pneumoniae* to overcome the mucosal immune defenses in the URT and travel to the middle ear, leading to otitis media (OM). Using a novel disease model for pneumococcal OM, we show that infant mice co-infected with *S. pneumoniae* and influenza virus had significantly higher bacterial load in the middle ear, middle ear inflammation and hearing loss compared to mice colonised with *S. pneumoniae* alone. Furthermore, we show that it is the viral hemagglutinin (HA), and not the neuraminidase, that facilitates bacterial OM. Of the two HA subtypes currently circulating in the human population, only H3 viruses induced disease, which reflected their ability to induce inflammation in the middle ear.

We then sought to use the observation that influenza virus induces bacterial replication in the middle ear to study the role of different adjuvants in mucosal vaccination against pneumococcal OM. Here, we show that mucosal vaccination with pneumococcal surface protein A in combination with cholera toxin subunit B (CTB) protected mice against influenza virus-induced replication of *S. pneumoniae* in the middle ear cavity and to a lesser extent in the nasopharynx. Mice vaccinated with CTB and PspA showed significantly stronger IgA responses compared to other adjuvants. Our findings strongly emphasize the value of using well-defined, clinically relevant animal models of disease for vaccination purposes, in particular for complex multi-factorial diseases such as pneumococcal OM.

O072

Phylogeny of European *Bordetella pertussis* and the occurrence of vaccine antigen deficient (VAD) mutants

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Introduction: Despite a long vaccination history, whooping cough is still prevalent in Europe and has even resurged in the 1990s. The causative agent of whooping cough is *Bordetella pertussis*, a highly monomorphic bacterium. Several studies suggest pathogen adaptation is one of the contributing factors to the persistence of *B. pertussis*. Since the introduction of vaccinations, an accumulation of

small mutations has been observed, mostly in virulence-associated genes. Some of these mutations have been associated with clonal expansions of *B. pertussis* suggesting they affect fitness. A striking recent observation is the emergence of strains which do not produce one or more vaccine components (designated VAD mutants). In Europe, vaccination programmes are organized nationally, each having their own composition of vaccine components and vaccination schedule. The genes coding for antigens present in a vaccine are likely under selective pressure due to vaccination. The genetic and phenotypic characteristics of the bacteria circulating in different countries may allow the elucidation off the effect of different vaccination strategies.

The persistence and adaptive capabilities of *B. pertussis* necessitates the need for surveillance. It also calls for reliable methods to analyze the circulating strains so changes in the population can be followed and possibly traced back to the influence of the different vaccination strategies.

Here we present the result of a SNP (Single Nucleotide Polymorphism) analysis on a collection of recent European *B. pertussis* strains, containing a few known VAD mutants. Also, an assay to screen for mutants non expressing vaccine antigens was developed and tested on a subset of the collection.

Methods: To establish the population structure of B.pertussis in Europe, 374 strains were typed using 117 SNPs. These strains were derived from a total of eight European countries isolated between 1998 and 2011. Further, a subset of the most recent strains (from 2004 onwards, n = 194) was used to explore the presence of VAD mutants. For this, the expression of the vaccine components pertussis toxin (Ptx), pertactin (Prn) and filamentous hemagglutinin (FHA) was measured by a microsphere-based multiplex Immuno Assay.

Results: 1. The phylogenetic analysis showed a closely related, recently evolved population of European *B. pertussis* strains with little geographic structure. The latter suggests a high strain flow between strains across Europe. 2. Nineteen VAD mutants from five European countries were identified. These strains were either deficient in the vaccine components Prn or FHA, or both. 3. The VAD mutants were randomly distributed within a young branch, implying a recent origin. As yet, no evidence was found for clonal expansion of the escape mutants.

Conclusion: 1. The lack of geographic structure in the European Bordetella pertussis population indicates high strain flow across Europe. As yet, there is no evidence that the difference in vaccination strategies among European countries influences the population structure. 2. Our data suggests that VAD mutants arose recently in Europe 3. VAD mutants have arisen independently in distinct branches. This homoplasy suggests that the mutations affect strain fitness. Further studies are required to assess the effect of the VAD mutations on vaccine efficacy.

O073

In search for novel pertussis vaccine targets: a comprehensive transcriptomic and proteomic approach

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Introduction: Pertussis, or whooping cough, is a highly contagious, acute respiratory disease of humans that is caused by the gram-negative bacterial pathogen *Bordetella pertussis*. There is need for improved pertussis vaccines, as pertussis has resurged in the face of intensive vaccination. The pertussis resurgence in the Netherlands has coincided with the emergence of *ptxP3* strains carrying a mutation in the promoter region of the pertussis toxin (Ptx) operon that is associated with higher levels of Ptx expression. Within a timeframe of 10 years, these strains nearly completely replaced the previously dominant *ptxP1* strains. Significantly, *ptxP3* strains have recently spread globally. During infection, the transcription of nearly all virulence genes is controlled by a two-component regulatory system designated the Bordetella master virulence regulatory system (*bvgASR*). *In vitro*, this system can easily be activated (Bvg^+), intermediately suppressed (Bvg^i), or fully suppressed (Bvg^-) by adding 0, 5, or 50 mM $MgSO_4$ to the culture medium respectively. Here, we used a multifaceted approach of transcriptional profiling in combination with label-free semi-quantitative nano-liquid chromatography-tandem mass spectrometry (nLC-MS/MS) to identify *bvg*-regulated proteins that are potential targets for improved pertussis vaccines. We were particularly interested in identifying genes upregulated in the *ptxP3* strains as this may elucidate how *B. pertussis* is able to thrive in highly vaccinated populations.

Methods: Two clinical isolates of *B. pertussis* (*ptxP1* and *ptxP3*) were grown *in vitro* under Bvg^+, Bvg^i, and Bvg^- conditions, after which RNA and protein were isolated. RNA samples were analyzed on custom-designed Nimblegen microarrays containing eight specific probes for each coding sequence (CDS) and pseudogene. The membrane proteins were separated from the cytosolic proteins and both fractions were subjected to *in-solution* tryptic digestion and subsequent label-free semi-quantitative nLC-MS/MS analysis using IDEAL-Q.

Results: Transcriptional profiling identified *bvg*-dependent differences in gene expression in both strains. An in-depth proteomic analysis allowed the identification of almost

1750 proteins, covering 68% of the observed transcriptome and 46% of the predicted proteome. For the majority of *bvg*-regulated genes a strong correlation between transcript and protein levels was observed. Interestingly, the BvgS sensor and BvgA transcription factor where both expressed at higher levels in the *ptxP3* strain under Bvg^+ and Bvg^i conditions respectively. Additionally, under Bvg^i conditions the *ptxP3* strain retained higher transcriptional and translational activity of type 3 secretion system (T3SS) toxin and Ptx, whereas the *ptxP1* strain expressed higher levels of adhesins and iron transport receptors. These datasets allow novel vaccine candidates to be selected and tested in mouse vaccination experiments.

Conclusions:

1. With almost 1750 identified proteins, this is the most comprehensive proteomic study of *B. pertussis* reported to date.
2. The transcriptional profile of *bvg*-regulated genes correlated strongly with the proteomic profile.
3. Compared to the *ptxP1* strain, the highly successful *ptxP3* strain showed elevated levels of T3SS toxin and Ptx expression.
4. The combined transcriptomic and proteomic analysis allowed us to identify surface-associated *bvg*-regulated proteins which may be potential components of future pertussis vaccines.

O074

Life threatening infections due to asplenia

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Patients with asplenia or hyposplenia are at risk for overwhelming infections. In absence of a (functional) spleen there is a significantly increased risk for invasive disease caused by encapsulated bacteria and certain intracellular parasites. *In absence of the spleen, encapsulated bacteria are not filtered from the circulation and can duplicate rapidly. Furthermore, since opsonizing antibodies are lacking, this process is mostly irreversible within hours.*

Post-splenectomy sepsis: The major risk associated with asplenia is the syndrome of post-splenectomy sepsis (PSS). This syndrome is characterized by a short and mild onset with flu-like symptoms; in hours rather than days a fulminant septic shock can develop. Mortality has been described to be as high as 70%, where 68% of patients die within 24 hours after the first symptoms.

Incidence: The incidence of PSS is estimated to be 2-5 per 1000 asplenic patients each year. The highest risk is associated with splenectomy due to hematological malignancies, in patients with a hemoglobinopathy and in children under the age of 5. It has been reported that over 50% of overwhelming infections occurs within the first 2 years after splenectomy, but the risk remains increased lifelong.

Microbiology: Encapsulated bacteria are the most important causative organisms of PSS. *Streptococcus pneumoniae* causes 70% of bacteraemic episodes. Other pathogens associated with PSS are *Haemophilus influenzae*, *Neisseria meningitidis* (meningococcus). *H. influenzae* as has become a less common microorganism of PSS, since Hib-immunization was added to the vaccination programs. Mortality associated with Hib is estimated to be lower than with pneumococci (37%). Other severe infections that are associated with asplenia are caused by *Capnocytophaga canimorsus*, an anaerobe microorganism causing wound infections after dog- or catbites, *Plasmodium falciparum*, causing malaria, and babesiosis which is transferred through tick bites.

The syndrome of PSS can be prevented if simple measures are taken, such as immunizations and prophylactic early use of antibiotics.

O075

A new LCI guideline for prevention of infections in patients with hypo- and asplenia

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In the Netherlands, patients after splenectomy are not managed according to best practice. Although vaccination rates are comparable to international figures, only 30% of patients receive all 3 recommended immunizations, and prophylactic antibiotics are prescribed only to a minority of patients. Furthermore, patients are not adequately educated about the risks associated with asplenia.

Recommendations: Although preventive measures are described in literature repeatedly, to date there has been no national guideline for these patients. The taskforce WIHA (Werkgroep Infectie-preventie bij Hyposplenie en Asplenie) has composed recommendations in collaboration with the LCI (Landelijke Cordinatie Infectieziektebestrijding), to prevent overwhelming infection in patients with (functional) asplenia. In the absence of clinical evidence, these recommendations are based mainly on case studies and expert opinion.

Preventive measures are threefold:

1. Immunizations. All asplenic patients should be immunized against the encapsulated bacteria *Streptococcus pneumoniae*, Haemophilus influenzae and Neisseria meningitidis. Furthermore, they should receive annual influenza vaccinations to prevent secondary bacterial infection.
2. Antibiotic therapy. Both prophylactic therapy (continuously during the first 2 years after splenectomy) as well as 'on-demand' antibiotics to use in case of (suspected) infection.

3. Patient education on the risks associated with asplenia and traveling.
These recommendations will appear on the website of the RIVM:
www.rivm.nl/Onderwerpen/Ziekten_Aandoeningen/A/Asplenie

O076
Efficacy of vaccinations after splenectomy
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One of the most important measures to prevent an overwhelming post-splenectomy sepsis (PSS) is vaccination against the encapsulated bacteria that cause these life-threatening infections. Vaccines recommended for asplenic patients target the encapsulated bacteria *Streptococcus pneumoniae*, *Neisseria meningitidis* (in particular serogroup C) and *Haemophilus influenzae type B* (*Hib*).
For *Streptococcus pneumoniae* both a conjugated (10- or 13-valent) and a non-conjugated 23-valent polysaccharide vaccine are recommended. The conjugated vaccine induces immunological memory and can be given once. More than 80% of the asplenic patients reach adequate antibody concentrations after one dose of conjugated vaccine. The 23-valent vaccine does not induce memory and should be repeated at least once after 5 years.
For both *Neisseria meningitidis* and *Hib*, conjugated vaccines are used with response rates of 97% and 67%, respectively. Both vaccines induce memory and can be given once.
For *Neisseria meningitidis* a serogroup C vaccine is given. In case of travelling to endemic countries for meningococcal disease, additional vaccination against serogroup A, W and Y can be considered.
Moreover, the influenza virus vaccine is recommended every year, in order to prevent a bacterial super-infection after a viral upper airway infection caused by the influenza virus.

O078
Genetic susceptibility to viral Lower Respiratory Tract Infections (LRTI) in Europe
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Lower Respiratory Tract Infection (LRTI) is one of the leading reasons for seeking primary medical care in Europe. However, not everyone is equally susceptible to LRTI. A large body of evidence shows that there is a genetic basis for susceptibility to infectious disease. A number of rare mutations causing primary immunodeficiencies as well as common genetic variants predisposing to infectious diseases have been reported. Some of the best known examples include CCR5fj32 polymorphism that modulates HIV-1 disease progression and the beta haemoglobin mutation that protects against malaria. However, host genetics of mild, very common infections such as LRTIs have not been studied on a large scale before.
We have conducted a study to identify genetic factors that may play an important role in explaining this inter-individual variation in susceptibility to LRTI within GRACE (GRACE; Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe, a Network of Excellence focusing on community-acquired Lower Respiratory Tract Infections. 3000 adult patients with acute cough or clinically suspected LRTI as the main presenting symptom and 3000 matched controls were recruited by 14 primary care research networks in 12 European countries, making this the largest prospective study of LRTI ever undertaken in primary medical care. Owing to the detailed microbiological characterisation within the GRACE study, we were able to analyse non-viral and viral LRTIs separately, and further divide the latter to specific viruses. Interestingly the most convincing associations between the host genetic polymorphisms and lower respiratory tract infections were seen within the viral infection subgroup, and even more so among patients with Rhinovirus infection. In my talk, I will describe these genetic findings and present possible mechanisms underlying these associations.
Identification of these genetic factors will hopefully increase knowledge about biology and pathways involved in LRTI disease process and might thereby create new targets for drug development and individualised treatment.

O081
The sigma factors of *Mycobacterium tuberculosis*
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Mycobacterium tuberculosis is a remarkable pathogen capable of adapting and surviving to various harsh conditions encountered during infection. It can also enter a dormant state, allowing asymptomatic infections that persist for decades. Any weakening of the immune system response, possibly due to malnutrition, debilitating diseases or age may result in the reactivation of latent bacilli.
The sophisticated infection and adaptation mechanisms used by *M. tuberculosis* are expected to require complex genetic programs. The genome of *M. tuberculosis* is the largest among obligate human pathogens and intracellular bacteria, and encodes approximately 190 regulatory

proteins, including 11 two-component systems, five unpaired response regulators, two unpaired histidine kinases, 11 protein kinases and over 140 other transcription regulators, including 13 sigma factors. Most of them have been investigated, and so far were all shown to be required for virulence. The better characterized alternative sigma factor of *M. tuberculosis* is SigE, belonging to the extra-cytoplasmic functions (ECF) sigma factors. It can be transcribed from three different translational start codons and is subjected to a very complex regulatory circuit including three positive forward loops involving the two component system MprAB, the antisigma factor RseA, the serin-threonine protein kinase PknB, the global regulator ClgR and the ECF sigma factor SigH. Beyond being responsible for controlling surface stability and composition following the exposure to damaging environmental conditions, its function confers ability to avoid the induction of the host response. A *sigE* mutant of *M. tuberculosis* is unable to grow inside macrophages and is severely attenuated in mice. Interestingly, this mutant confers better protection than *M. bovis* BCG from infection with virulent *M. tuberculosis*.

O082

Alternative sigma factor regulation in pseudomonas

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Gene expression in bacteria is mainly controlled at the level of transcription initiation. There are different mechanisms to achieve this control, one of which is the utilization of alternative sigma factors. Sigma factors bind to the core enzyme of the RNA polymerase and direct it to specific promoter sequences initiating transcription. Bacteria regulate transcription initiation by producing and activating different sigma factors that recognize different promoters. The largest group of alternative sigma factors consists of the so-called extracytoplasmic function (ECF) sigma factors that regulate gene expression in response to cell envelope stresses or environmental stimuli. Bacterial species with large genomes living in complex habitats are known to contain many ECF sigma factors. This is the case for the versatile opportunistic pathogen *Pseudomonas aeruginosa*, which encodes 19 ECF sigma factors. By genomic and proteomic approaches we have determined that most *P. aeruginosa* ECF sigma factors are involved in the regulation of iron uptake. One *P. aeruginosa* ECF sigma factor controls the uptake of the endogenous siderophore pyoverdine, and at least eight ECF sigma factors regulate iron uptake via heterologous siderophores (such as ferrioxamine B, ferrichrome or (carboxy)mycobactin), haem or iron citrate. Furthermore, another ECF sigma factor controls the production of pyocins, probably in response to the presence of a specific siderophore. Interestingly, this bacterium also contains two ECF sigma factors that control the production of virulence factors. One of these regulates the production of the endogenous siderophore pyoverdine (which itself is a virulence factor), an exotoxin, and an endoprotease. The other one, named VreI, controls the expression of genes encoding secreted proteins and components of secretion systems. Constitutive VreI activity significantly increased the virulence of *P. aeruginosa* in the zebrafish embryo infection model. Moreover, the serum of most *P. aeruginosa* infected patients contains antibodies directed against VreI-regulated proteins, which indicates that the VreI regulon genes are transcribed *in vivo* during infection. Apparently, VreI is activated by a host signal, which is consistent with previous reports showing that interaction of *P. aeruginosa* with human airway epithelial cells induces the expression of many VreI-regulated genes.

O083

Promoter propagation in prokaryotes

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Introduction: Transcriptional activation or 'rewiring' of silent genes is an important, yet poorly understood, phenomenon in prokaryotic genomes. However, anecdotal evidence coming from experimental evolution studies has shown that, upon selection pressure, silent genes can become rapidly activated. Closer inspection of the mutations leading to transcriptional activation has revealed that in most cases a partial or complete promoter has been mobilized to the silent gene from elsewhere in the genome. Furthermore, studies on DNA repeat families have also shown the extensive distribution of repetitive intergenic DNA and its role in regulating gene transcription.
Methods: Given the potential importance of promoter propagation in adaptive evolution, we set out to investigate its prevalence in all published prokaryotic genomes, excluding known mobile elements such as insertion sequences. We compared the intergenic sequences per genome for intragenomic duplication events, and enumerated the number of clusters and cluster size per genome. Next, we tested for sequence similarities between clusters in different species in order to identify intergeneric transfer events.
Results: About 2000 clusters of highly conserved duplicated promoters (50 to 100-bp long with> 80% nucleotide identity) were identified in over 800 phylogenetically diverse genomes. The largest number of distinct clusters was identified in the cyanobacteria *Nostoc punctiforme* (26 clusters), *Microcystis aeruginosa*

(24 clusters), *Trichodesmium erythraeum* (22 clusters). The largest cluster was found in Treponema brennaborense (ten sequences), with the highly conserved promoters preceding a range of functional genes in the genome. Most clusters however consist of only two elements, with a mean nucleotide identity of 94%. Clusters are mainly present in a single genome only (singletons), although we also detected 195 homologous clusters between different strains of the same species, another 108 homologous clusters between different species of the same genus, and 54 homologous clusters between representatives of different genera.

Conclusion: We conclude that most promoters in our dataset have recently been duplicated and may be diverging rapidly, based on the predominant small cluster size and high mean nucleotide identity. Also, clusters that are homologous between representatives of different genera suggest recent transfer events. Variations in cluster sizes between strains of the same species hint at diverse turnover rates of these regulatory elements. Together, the identified mobile promoters represent a conservative dataset of very recent or conserved events of transcriptional rewiring, and allow further investigations into the different mechanisms that result in the propagation of regulatory elements.

O084

A novel cell-surface signalling system uncovers the intimate relationships between the components of this signalling cascade

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Cell-surface signalling (CSS) is a mechanism by which gram-negative bacteria regulate gene expression in response to extracellular signals. Most of these systems are involved in the regulation of iron uptake, although also other (virulence) factors can be induced. CSS systems generally consist of an outer membrane receptor, a sigma factor regulator (SFR) in the cytoplasmic membrane, and an alternative sigma factor of the ECF σ^{70} subfamily. Perception of the extracellular signal by the receptor ultimately results in activation of the sigma factor, upon which it associates with the bacterial RNA polymerase and promotes the transcription of a specific set of genes. However, the molecular mechanism by which the signal is transduced by the SFR is not completely understood. In the current model, the SFR retains the sigma factor at the cytoplasmic membrane in an inactive form in absence of the signal. Only in presence of the signal the SFR is activated through an interaction with the receptor, upon which the sigma factor is released into the cytosol. The dissociation of the ECF sigma factor from the SFR is thought to be controlled by protease(s).

To get more insight in the molecular working mechanism of CSS we focus on a unique, newly identified hybrid gene of *Pseudomonas putida* that encodes a chimeric protein combining both an ECF sigma factor in the N-terminal part and a SFR in the C-terminal part. In this case a fixed interaction between the sigma factor and the SFR is present and signalling needs to modify or abolish this interaction. Our analyses show that this gene indeed encodes a functional hybrid protein of around 40 kDa. Removal of the SFR domain of the protein resulted in constitutive activity of the sigma factor domain. We have also identified that this protein is activated by the presence of the *Escherichia coli* siderophore aerobactin in low iron conditions. Subsequently, we set out to identify putative other factors involved in the regulation of this system. Using a transposon mutant bank of P. putida we identified a cytoplasmic membrane protease required for the aerobactin-mediated activation of the sigma factor. This would indicate that the sigma factor is activated by protease cleavage. However, analysis of an HA-tagged protein by SDS-PAGE showed no proteolysis of this hybrid protein in the presence of aerobactin. Interestingly, the introduction of an HA-tag in the C-terminal end of the SFR domain resulted in constitutive activity of the sigma factor. Based on these results we propose a new model in which the interaction between the receptor and the SFR occurs in the absence of aerobactin and is required to maintain the sigma factor in its inactive form. The presence of aerobactin, or the introduction of an HA-tag in the C-terminal end of the SFR domain, disrupt this interaction and produces the activation of the sigma factor probably through a conformational change of the hybrid protein. The identified protease may be required to remove the receptor-SFR interaction in the presence of aerobactin.

O085

Differentiation in multicellular *Bacillus subtilis* communities

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The soil-dwelling bacterium *Bacillus subtilis* is able to form multicellular communities known as biofilms. Cells that constitute the biofilms of *B. subtilis* are embedded in an extracellular matrix that held cells together and gives consistency to the microbial community. Biofilm communities of of *B. subtilis* are composed by cells, which differentiate into distinct specialized subpopulations. These specialized cells express a particular set of genes that provide physiological trait to each subpopulation and

make them different from the rest, resulting in a division of labor of the members of the microbial community. Cell differentiation in biofilms of *B. subtilis* relies on the activity of three major regulatory proteins, Spo0A, DegU and ComA. Each phosphorylated regulator triggers a specific differentiation program. The activation of the regulatory proteins occurs in a bistable manner and it is mediated by an extensive extracellular communication process that is mostly driven by sensing self-generated secreted signals. This presentation will show how cell differentiation occurs in *B. subtilis* in respond to the production of self-generated signaling molecules but also by the presence of natural products secreted by other soil-dwelling microorganisms. These small molecules are sensed by a set of sensor kinases that are able to activate one of the three master regulator while repressing the expression of the other regulators. This mechanism guarantees that cell differentiation occurs in response to a specific cue, even in the presence of other conflicting signals. Remarkably, some of these sensor kinases were recently found to localize in functional membrane microdomains that are similar to the so-called lipid rafts of eukaryotic cells. The integrity of the bacterial lipid rafts is essential to maintain the functionality of certain sensor kinases and therefore, are essential to induce the differentiation of certain cell types within *B. subtilis* multicellular communities.

O086

Heterogeneity in the fungal mycelium

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Filamentous fungi secrete large amounts of proteins into their environment to degrade organic substrates. The degradation products are taken up by the fungal hyphae to serve as nutrients. The enormous secretion capacity of filamentous fungi is used by the industry to produce enzymes of industrial and pharmaceutical interest. Notably, productivity is very heterogeneous between and within mycelia of the filamentous fungus *Aspergillus niger*. *A. niger* forms (sub)millimeter micro-colonies within a liquid shaken culture. We have shown that such micro-colonies are heterogenic with respect to size and gene expression. Micro-colonies of strains expressing GFP from the promoter of the glucoamlyase gene glaA or the ferulic acid esterase gene faeA were sorted on basis of diameter and fluorescence using the Complex Object Parametric Analyzer and Sorter (COPAS). Statistical analysis revealed that the liquid shaken culture consisted of two populations of micro-colonies that differ 90 m in diameter. The population of small micro-colonies of strains expressing GFP from the glaA or faeA promoter comprised 39% and 25% of the culture, respectively. Two populations of micro-colonies could also be distinguished when expression of GFP in these strains was analyzed. The population lowly expressing GFP consisted of 68% and 44% of the culture, respectively.

Heterogeneity with respect to gene expression, growth and secretion is also observed within a mycelium. For instance, only part of the hyphae at the periphery of the mycelium of *A. niger* secrete glucoamylase. This is due to heterogeneous expression of glaA in this part of the colony. In fact, two populations of hyphae can be distinguished; those that highly and those that lowly express glaA. Hyphae that show high glaA expression also highly express other genes encoding secreted proteins. Moreover, they have a high rRNA content and highly express the glyceraldehyde-3-phosphate dehydrogenase gene gpdA. From this it was concluded that two populations of hyphae can be distinguished at the outer part of the vegetative mycelium; those with a 'high' and those with a 'low' transcriptional and translational activity. The low activity would be sufficient to support growth but a high activity would be needed to support secretion of high amounts of protein.

To further explore the concept of hyphal heterogeneity we performed a whole genome expression analysis on 5 individual neighboring hyphae. The picogram amount of RNA of single hyphal tips was amplified to microgram quantity cDNA. Quantitative PCR on this cDNA showed that levels of 18S rRNA, and of RNA of the actin gene and the glucoamylase gene glaA were heterogeneous between the neighboring hyphae. Microarray analysis resulted in a present call for 4-7% of the *A. niger* genes, of which 12% showed heterogeneous RNA levels. These genes belonged to a wide range of gene categories, among which metabolism, transcription and rRNA and tRNA.

At the moment we are studying the mechanisms underlying hyphal heterogeneity. Understanding these mechanisms may enable us to improve protein production in *A. niger*.

O087

Common genes are required for architecturally complex colony formation and sliding motility of *Bacillus subtilis*

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Bacteria colonize surfaces in various ways. When growing on semi-solid surfaces, various strains of *Bacillus subtilis* show swimming, swarming, and sliding motility depending on the agar concentrations. While swimming

and swarming depend on the activity of flagella, sliding is a passive surface translocation and does not require an active motor. On rich medium with higher agar concentration *B. subtilis* forms architecturally complex colonies. Several regulators were described to affect both motility behaviors and biofilm formation in *B. subtilis* [1]. Depending on the environmental conditions and cell-cell communications, the intertwined regulatory pathways determine the differentiation into the various phenotypes of the cells within the community.

Examination of strains with reduced biofilm structure formation in *B. subtilis* resulted in the identification of *yuaB* gene required for biofilm development [2]. YuaB is small protein that upon secretion localizes to the cell wall and functions synergistically with the known components of the biofilm matrix to facilitate the assembly of the biofilm matrix [3]. Transcription of *yuaB* is regulated by several global regulators and shows a novel spatiotemporal expression pattern during the development of biofilm. It is expressed early during biofilm development and localized to the edge of the matured complex colonies. Interestingly, we identified *yuaB* in our recent microarray experiments where we examined the sliding behaviour of *B. subtilis* Natto under sliding restrictive compared to permissive conditions (using *spo0A* mutant strain or higher agar concentration). Introduction of the *yuaB* mutation into *B. subtilis* Natto reduced sliding motility.

Our results point to presence of shared regulators and genes for distinct surface-dependent growth of *B. subtilis* that determine spatial gene expression and differentiation in multicellular communities.

References

1. T Abee, AT Kovacs, OP Kuipers, S van der Veen 2011 *Curr Opin Biotechnol* 22(2) 172-179
2. AT Kovacs and OP Kuipers 2011 *J Bacteriol* 193(4) 998-1002
3. A Ostrowski, A Mehert, A Prescott, TB Kiley, NR Stanley-Wall 2011 *J Bacteriol* 193(18) 4821-4831

O088

Spatially resolving the secretome within the mycelium of the cell factory *Aspergillus niger*

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Fungi secrete enzymes to convert organic matter into small molecules that can serve as nutrients. Hyphae at the periphery of the colony are exposed to unexplored organic material, whereas the center of the colony experiences a utilized substrate. This suggests that the enzymes that are secreted by different zones in the colony are different. *Aspergillus niger* is an important cell factory for the industrial production of enzymes. Here, we determined with stable isotope dimethyl labeling the secretome of 5 concentric zones of 7-day-old xylose-grown colonies of *A. niger* that had either or not been treated with cycloheximide. As expected, cycloheximide blocked secretion of proteins at the periphery of the colony. Unexpectedly, protein release was increased by cycloheximide in the intermediate and central zones of the mycelium when compared to non-treated colonies. Electron microscopy indicated that this is due to partial degradation of the cell wall. A total of 124 and 59 proteins were detected in the medium of xylose grown colonies that had or had not been treated with cycloheximide. Three proteins were only detected in the absence of the antibiotic, whereas 67 proteins had not been identified in non-treated colonies. Apparently, a major part of the proteins are associated with the cell walls of A. niger. Notably, all 16 secreted proteins encoded by XlnR regulated genes were detected after cycloheximide, while only 13 were found in the untreated secretome. Proteases, cellulases, hemicellulolytic enzymes, and enzymes with unknown function were among the other proteins that were only detected after treatment with the antibiotic. A total of 70 and 65 proteins were ≥ 4-fold more abundant in the central zone 1 and the intermediate zone 3, respectively, in cycloheximide treated colonies when compared to untreated colonies. In contrast, only 4 proteins were ≥ 4-fold more abundant in the outer zone 5. The latter is explained by the fact that the mycelium in zone 5 of non-treated colonies could continue growth and secretion, while cycloheximide treated colonies could not. Taken together, cycloheximide can be used to obtain a (near) complete secretome of *A. niger*. Moreover, the total amount of protein is increased upon treatment with this antibiotic. The composition of the secretome in each of the 5 concentric zones differed. This study thus describes spatial release of proteins in *A. niger*, which is instrumental in understanding how fungi degrade complex substrates in nature.

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O089

Social media and microbiology education

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Social media is the part of the Internet where the content is generated by users of the service rather than conventional publishers. Such content ranges in scope from

short comments on blogs, status updates on social networks and 140 character "tweets", to lengthy blog posts sometimes even containing original research. In comparison to conventional academic publishing, the social media landscape is extremely varied. Although the age demographic of social media users is becoming older and more inclusive, the typical social media user is aged 18-30, spends more time online and gaming than watching television, and gains a much higher proportion of their information by searching and social recommendations than through traditional publishing channels. Social media is the backbone of their information infrastructure. This talk will address the following questions:

- What does the current generation of students want?

Their problem is not shortage of information but overabundance. They intuitively expect academics to compete for their attention with professional media such as the games industry and Hollywood. In an educational context, they want guidance and leadership through the information maze – academic mentors. In the current environment, they also want value for money and a return on their investment, both financial and of their time.

- What do we give them?

By and large, we give them what we ourselves experienced in education. Where technology makes it easy for us to increase the pressure on them (by email, online assessment), we do so. When we venture online, we expect them to use information on our terms, not theirs. We wedge them into virtual learning environments planned and built when the Internet was young, when they were still infants and before social media existed.

- What do we (academics) want?

We want highly engaged, enthusiastic, self-motivated, lifelong learners who will go on to successful and profitable careers. We want the satisfaction of seeing students gradually awake to an understanding of the subject we love and have spent our careers working on. We want students to look to us for help, support advice and guidance.

- So how do we get there?

We need to invest much more time and effort in understanding how new media work rather than putting our PowerPoint slides online. We need to manage expectations – in particular that education is an active process, not passive spoon-feeding of information. We need to give students clear targets and something to aim for. And we need to engage with student attention in social media to achieve these aims.

References

- Junco, R. (2012) The relationship between frequency of Facebook use, participation in Facebook activities, and student engagement. Computers & Education 58(1): 162-171.
- Kanai, R., Bahrami, B., Roylance, R., & Rees G. (2011) Online social network size is reflected in human brain structure. Proceedings of the Royal Society of Biological Sciences. http://rspb.royalsocietypublishing.org/content/early/2011/10/12/rspb.2011.1959.abstract.
- Mollett, A., Moran, D. & Dunleavy, P. (2011) Using Twitter in university research, teaching and impact activities: A guide for academics and researchers. LSE Public Policy Group. http://blogs.lse.ac.uk/impactofsocialsciences/files/2011/11/Published-Twitter_Guide_Sept_2011.pdf.
- Racaniello, V. (2010) Social Media and Microbiology Education. PLoS Pathogens 6, 10, e1001095.
- Rainie, L. (2011) The internet as a diversion and destination. Pew Internet http://pewinternet.org/Reports/2011/Internet-as-diversion.aspx.

O090
Distance learning

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With the expanding technical possibilities of Internet, distance learning has become a widespread way of teaching in many educational institutes. Increasingly computer based learning elements are applied that allow students to learn without any teacher or teaching assistant present. Without the necessity of attending meetings in a predetermined teaching schedule, distance learning allows students to study at their own pace, in their favourite location, and at a time that suits the individual student.

Distance learning does not have a fixed format. All kind of varieties have been developed. Activities for the individual student include reading, answering closed questions, writing an assignment, simulating, and even gaming. In addition a number of tools have been developed to facilitate and stimulate collaboration among students that are physically separated from each other, everyone at their own computer.

The baseline for any type of distance learning course is that it is transparent for the participant. During the development of distance learning material relatively a lot of attention should be paid to meta-information and to issues that students 'on-site' are less likely to encounter, such as: 'How will I access course materials?, and 'How and when will somebody support me?'.

All this information should be close to flawless. If a page number is wrong in teaching material for on-site use, a teaching assistant will be able to quickly guide the students to the correct page. When stumbling upon an incorrect page number in distance learning material, however, students may get completely lost, and more importantly, may get demotivated.

Motivating the students is key to successful distance learning. Distance learning material requires incentives for participants to start, to persevere, and to complete the course. An important element In motivation is examination. In the presentation a variety of distance learning materials in microbiology and related areas will be shown.

O091

Social media and infection control: trending topic?

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Control of infectious diseases, especially during large-scale epidemics or incidental high risk outbreaks, increasingly suffers from deficient compliance with preventive measurements or guidelines of both professionals and general audience.
Examples are the no-show rate among the general audience after receiving a personal appeal to obtain an influenza or HPV-vaccination, or similar low adherence among health care workers with preventive measures such as wearing protective gear (like masks, gowns and protection glasses) during professional care for potentially infectious patients, low influenza vaccination or vaccin uptake among health care workers (to protect their patients for Hepatitis B or Influenza). This poses a threat to public health in the short term.
The RIVM Centre for Infectious Disease Control (CIb) has an executive and coordinating task in the national prevention and control of a wide range of infectious diseases manifesting themselves in incidental outbreaks of mixed origin, scale and risk level, as well as various epidemics. This is one of RIVM's most important and most visible statutory public assignments.
In order to be able to effectively implement the national infectious disease control policy in the near future it is of strategic interest to counter the above mentioned health risks. We know from social health sciences what psychological, social and cultural factors influence nonadherence. We also know that current approaches to prevent risk behavior are expensive, tired and hardly effective. Fresh approaches are needed. We find these in eHealth marketing; a sub domain of health and social marketing. eHealth marketing concerns public health practice. It draws from traditional marketing theories and principles, and adds evidence-based strategies to prevention, communication, health promotion and health protection on a wide range of topics. In infectious disease control, where time is often a key determinant of success as immediate implementation is a prerequisite for proper control, there is little or no experience with these techniques. eHealth provides a framework of theories, strategies and techniques that can be used to guide work in public health research, interventions, and communication campaigns. eHealth marketing typically uses emerging technologies and digital media' to improve the impact of health marketing and communication. Web-based and mobile technologies offer tools that are cheap, ubiquitous, interactive, real-time, many-to-many and participative in nature. They can be put to action for infection control objectives to make content, tools and services available when, where and how users want them.
We are busy to design, operate and evaluate a limited number of social media tools according to a participatory health care design approach in order to increase adherence to preventive measures and guidelines in infection management.
Both a professional and a general audience are targeted.
In the lecture some current social media experiments with infection control in public health will be presented.

O093

Blended learning in laboratory education

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Since 2005 laboratory education of ROC Midden Nederland in Utrecht uses the online learning environment of Blackboard to support lessons. Blackboard is used as a communication and information medium. Students can find announcements and study guides and deliver their assignments digitally. After 5 years an intensive didactical use of Blackboard does not exist. An important reason for this was the lack of ICT workstations at school. Therefore two years ago the school conducted the mandatory use of laptops: all students must bring their own laptop to school. Education at the laboratory school in Utrecht is presented in a blended' form: laboratory practice interspersed with blackboard, textbook and laptop. On the laptop students practice math and language, do digital exercises on physics, chemistry and biology, and both the preparation of the laboratory experiments and the processing of the results is on the laptop. Students are working with the laptop supervised in the classroom, and independently outside the classroom.
Teachers will benefit from the use of laptops in the classroom. It can easily be switched between teaching methods with blackboard, textbook and the Internet. Online exercises are more attractive than textbook assignments. The students have made assignments on current topics such as the cholera epidemic in Haiti, EHEC in Germany, or Klebsiella in a Rotterdam hospital. Many assignments are multi-media including YouTube video instructions which train laboratory techniques. Online tests and quizzes are interactive and give automatic feedback on the learning process.
Students with laptops also offers advantages for the school. Experience in education shows that school computers are not handled gently by students. Laptops with broken screens are no exception. But with their own laptop it's much different. Students' laptops are up to date and always works.
The ICT infrastructure in libraries and computer labs can be largely dismantled and replaced by a wireless network. ROC Midden Nederland is connected to Eduroam which

students can use to access the Internet. There are sufficient access points, so many students can browse on the Internet simultaneously.
In the initial evaluations, students work more independently with their laptops. Students work outside the lessons on their assignments, or do the online assignments at home. The Laboratory School has scheduled some online assignments outside the 'traditional' lessons. Teachers can 'remotely' control the digital lessons, because the assignments are automatically administered in Blackboard. Because group size hardly plays a role in digital lessons, teaching time is more effectively utilized. The time saved is used by teachers for individual support for students with learning disadvantages.
Eus van Hove will share in this presentation his experiences with blended learning in laboratory education and will show some examples of his online teaching microbiology.

O094

The role of genomic islands in the cross-kingdom spread of enteric pathogens

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Introduction: Bacteria belonging to the family *Enterobacteriaceae* (enteric bacteria) live in a wide range of habitats and include both human/animal (e.g. *E. coli* and *Salmonella*) and plant pathogens (e.g. *Pectobacterium* and *Dickeya*). Plants are increasingly implicated in the spread of human infections, yet little has been done to understand the interactions between them.
Pectobacterium atrosepticum (*Pba*) is a pathogen of plants and is taxonomically related to the enteric human pathogens. Our work sets out to discover "what makes an enterobacterial plant pathogen?" and "are *E. coli* and *Salmonella* strains able to colonise and infect plants as part of their spread through the environment to infect humans/animals?"
Methods: We sequenced the genome of *Pba* strain SCRI1043, in collaboration with the Sanger Centre, and developed a tool to compare multiple bacterial genomes. This tool was later refined to help identify genes within the *Pba* genome that may have been acquired from other plant-associated bacteria. The work was followed by the development of a mutation library for rapid isolation of mutants to test the role of these genes in pathogensis and other aspects of the plant interaction. We also developed *Pba*-specific microarrays to examine gene expression during infection and to identify differences in genome islands.
Subsequent to this, we examined the role of genomic islands in the potential colonization and survival of *E. coli* and *Salmonella* on plants. We also used differences in genomic islands and other sequences to obtain strain/species -specific diagnostics for both human and plant pathogens using comparative genomics.
Results: From genome comparisons between *Pba* and other bacteria, *Pba* was shown to contain at least 17 islands, and mutation analysis revealed that these islands carry genes involved in both plant pathogenesis and other aspects of the bacterial-plant interaction. These islands were found to be twice as likely as the rest of the genome to contain pathogenicity-related genes with homology to those found in plant-associated bacteria, a high proportion of which were expressed during plant infection. However, surprisingly, there were considerable differences in the presence of genomic islands and their associated genes between *Pba* and closely related plant pathogens (*P. carotovorum* SCRI193 and *Dickeya dadantii* 3937). When we examined the possibility that *E. coli* and *Salmonella* may also contain genomic islands involved in plant colonisation, a number of islands were identified that contained genes with high homology to those found in plant-associated bacteria, and these are currently being investigated. Based partly on the differences in genomic islands between strains/species, we identified novel diagnostics for use in identifying and monitoring both plant and human enteric pathogens.
Conclusions: Genomic islands were found to play a major part in the life of *Pectobacterium* on plants and we have found some evidence that *E. coli* and *Salmonella* may also possess islands that allow them to colonise plants. Genomic islands were found to vary substantially between closely related plant pathogens, possibly reflecting their different modes of pathogenesis, a fact that we have exploited when designing strain and species-specific diagnostics.

O095

Barriers and bypasses to lateral gene transfer in prokaryotes

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Gene acquisition by lateral gene transfer (LGT) is an important mechanism for natural variation among prokaryotes. Laboratory experiments show that protein-coding genes can be laterally transferred extremely fast among microbial cells, inherited to most of their descendants, and adapt to a new regulatory regime within a short time. Recent advance in the phylogenetic analysis of microbial genomes using networks approach reveals a substantial impact of LGT during microbial genome evolution. Phylogenomic networks of LGT among prokaryotes reconstructed from completely sequenced genomes uncover barriers to LGT in nature at multiple

levels. These include physical barriers for gene transfer between cells, genomic barriers for the integration of acquired DNA, and functional barriers for the acquisition of new genes.

O096

Zoo animal gut microbiota as a reservoir of antibiotic resistance genes

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Introduction: The increasing prevalence of antimicrobial resistance in pathogenic bacteria represents a considerable health care issue for human and veterinary medicine, causing complications in the treatment of infections. Since antibiotic resistance determinants are thought to be exchanged easily and rapidly between bacteria through lateral gene transfer, there is an increasing interest in investigating reservoirs of antibiotic resistance genes. Indeed, various studies have demonstrated antibiotic resistance in cultured isolates from animal microbiota. The close association between human and (zoo) animals, promoted through activities such as feeding and handling of animals, has led to reports of zoonotic transmission of several mainly gastrointestinal strains. The aim of this study was to evaluate the prevalence of antibiotic resistant bacteria in faecal samples from zoo animals.
Methods: Twenty-one fresh faecal samples were obtained from 9 different species of exotic mammals kept at Burger's Zoo (Arnhem – the Netherlands) during the period September – November 2011. All animals were sampled once, by zoo keepers, and placed immediately in sterile collection containers. Only one animal was symptomatic at the time of sampling and was being managed clinically for diarrheal disease. Faecal samples were suspended in phosphate buffered saline, and ten-fold dilution series were inoculated on a range of nutrient agars (Brain Heart Infusion (BHI), Tryptone Soya Agar (TSA), Mac Conkey (MC), Eosine Methylene Blue (EMB), Bilis Esculin Agar (BEA), Colistin Nalidixic Acid (CNA)), with and without a pool of antibiotics, and incubated at 37°C for 24-72 hours under aerobic conditions. All colonies were characterized morphologically and by 16S rRNA gene sequencing. The antibiotic resistance profile as well as plasmid content was defined for all resistant isolates.
Results: Of 21 mammals, 34 gram-negative isolates were obtained from chimpanzees, gorillas, giraffes, elephants, monkeys, siamangs, tigers and warthogs; 29 isolates corresponded to *Escherichia coli*. Thirteen isolates showed multidrug resistance phenotypes, the most common being against: Ampicillin, imipenem, chloramphenicol, streptomycin and tetracycline. Molecular analyses identified 8 plasmids, ranging in molecular weight between 1.5 kbp and 3.0 kbp from a Chimpanzee, siamang, giraffe, tiger and warthog.
Conclusion: We have found that asymptomatic exotic mammals from the zoo carry populations of *Escherichia coli* with a broad range of antibiotic resistant strains. This study demonstrated the potential of animals as a natural reservoir of multidrug-resistant bacteria and antimicrobial resistance genes. Is important to consider that enterobacterial multidrug – resistance can be horizontally transmitted between bacteria in the densely populated animal intestinal tract, potentially leading to the emergence of highly virulent bacteria with multidrug – resistance.

O097

Functional metagenomic analysis reveals selection for antibiotic resistance in the gut microbiota during Intensive Care hospitalization

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Functional metagenomic screening of environmental DNA has revealed the existence of an enormous reservoir of antibiotic resistance genes in the soil and the human gut microbiota. Here we aimed to determine the dynamics of the antibiotic gene reservoir ('the resistome') in a patient who was hospitalized at the Intensive Care Unit (ICU) in our hospital and who received continuous antibiotic prophylaxis (Selective Digestive tract Decontamination; SDD) until ICU-discharge.
Four fosmid libraries (ranging from 0.8 GBp to 2.4 GBp in size) were constructed from fecal samples collected at three different time points throughout ICU stay (days 4, 14 and 16) and 11 days after discharge from the ICU. A strong selection for resistance to ampicillin could be observed during and after ICU hospitalization. Transposon mutagenesis and partial sequencing of the fosmids led to the identification of a β-lactamase gene in *Bacteroides* sp. In four of the nine analyzed clones, an IS element is present next to the identified β-lactamase gene, suggesting that the resistance gene could become mobilized.
In addition tobramycin- and tetracycline resistant clones were enriched in the first 2 weeks of ICU stay but decrease in number after ICU discharge. Partial analysis of the tobramycin resistant fosmid clones revealed that they originate from organisms belonging to the class Clostridia.

The monitoring of the gut microbiota composition of this patient using the Human Intestinal Tract Chip (HIT-Chip) revealed significant changes in the phylum and species composition during and after antibiotic treatment. Specifically a decrease in the relative abundance of *Bacteroidetes* and a concomitant increase in *Clostridium* species could be detected during ICU stay.
Our findings show strong selection for antibiotic resistance among anaerobes in a patient receiving prophylactic antibiotics during ICU stay. An increase of antibiotic resistance genes in the gut microbiota may enhance the possibility of resistance gene transfer to gut-dwelling opportunistic pathogens.

O098

Genetic identification and virulence contribution of the Group A streptococcal antigen

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Introduction: Group A streptococcus (GAS; *Streptococcus pyogenes*) is a preeminent human pathogen ranking among the top 10 infection-related causes of mortality worldwide. Rapid diagnostics of GAS infection relies on specific serological reactivity of group-specific carbohydrate antigens. The defining Group A carbohydrate (GAC) consists of a polyrhamnose core and an immunodominant N-acetylglucosamine (GlcNAc) side chain. No specific biological function has been attributed to the GAC. We hypothesized that this abundant and unique cell wall carbohydrate might have a role in streptococcal pathogenesis.
Methods: We coupled bioinformatics with allelic exchange mutagenesis (M1T1 strain 5448), lectin staining and glycoanalysis to identify the gene cluster responsible for assembly of the GAC. A possible role in virulence was determined by assessing survival of GAS wild-type and mutant bacteria in the presence of human whole blood, human neutrophils, serum and platelets. A rabbit infection model was used to study the role of GAC in streptococcal pathogenesis.
Results: Screening sequenced GAS genomes, we identified a 12-gene cluster that was rich in glycosyltransferases and polysaccharide transport proteins. Mutagenesis of gene gacI resulted in a viable "antigen negative"mutant that lacked the GAC GlcNAc side chain as demonstrated by lectin staining and glycoanalysis. f*igacI* mutant bacteria were avirulent in a rabbit infection model establishing the GAC GlcNAc side chain as a novel GAS virulence factor. Decreased virulence was linked to enhanced sensitivity of the f*igacI* mutant to human blood, neutrophil extracellular killing, specifically the cathelicidin antimicrobial peptide LL-37, and platelet-derived antimicrobial components present in serum.
Conclusion: 1) We have discovered and experimentally confirmed the genes involved in assembly of the historic Lancefield carbohydrate antigen of GAS. 2) The GlcNAc side chain of this unique antigen is a novel GAS virulence factor.

O099

A *Streptococcus pneumoniae* operon modifying interaction with host cells in a capsule-dependent manner

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Considering the disease burden caused by S. pneumoniae, the underlying host/pathogen interactions which govern the *S. pneumoniae* switch from extracellular commensal colonizer of the nasopharyngeal cavity, to invasive pathogen are still poorly understood. One proposed route of breaching cell barriers is via invasion and subsequent translocation of the host cells. Magnesium homeostasis and its regulation, has been shown to be of vital importance for the growth and full virulence of many pathogenic bacteria, in particular during the interaction with eukaryotic cells. Here we focused on a putative cell membrane associated operon consisting of an mgtC homologue and an ABC transporter. Analysis of its genomic region indicated the presence of a LacI-type regulator that might modulate its expression.
Deletion of the regulator resulted in increased transcription of the mgtC/ABC transporter operon as shown by transcriptome analysis. Deletion of the mgtC/ABC transporter operon, or the regulator, did not result in any substantial growth or viability defects in various conditions tested, including media depleted for magnesium and cations. In an unencapsulated background, neither deletion of the mgtC/ABC transporter operon, or the regulator, had any significant impact on adhesion to host cells, nor invasion of host cells. Interestingly, in an encapsulated background however, deletion of the mgtC/ABC transporter operon resulted in a substantial increase in both adhesion and invasion of the host cells. Deletion of the regulator resulted in a substantial reduction in adhesion but no subsequent reduction in relative invasion of host cells was observed. In accordance with the phenotypes observed in the adhesion and invasion assays, changes in the capsule of the mutants were observed.
Combined these results indicate that the mgtC/ABC transporter operon is under negative transcriptional control by the adjacent lacI type regulator. Furthermore,

we conclude that the mgtC/ABC transporter operon acts as a repressor of adhesion and invasion of host cells in an encapsulated background. We hypothesize that the operon might be implicated in the dynamics of colonization versus invasion of the host, possibly via capsule regulation.

O100

Pneumococcal immune evasion by Zinc metalloprotease C

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Introduction: *Streptococcus pneumoniae* is a commensal of the human upper respiratory tract but also a pathogen responsible for over a million deaths annually. The bacterium resides in the nasopharynx, and although colonization is asymptomatic, it can spread from this site and cause a wide range of diseases from otitis media, through pneumonia, septicemia and meningitis. The ultimate virulence factor of *S. pneumoniae* is the polysaccharide capsule and there are a number of surface proteins known to contribute to bacterium pathogenicity, relatively little is known about the role of secreted proteins in pneumococci virulence. In this study we tested whether pneumococci produce secreted proteins inhibiting leukocyte recruitment to the site of infection.

Methods: Human leukocytes were isolated from peripheral venous blood of healthy donors using a Ficoll/Histopaque gradient We isolated the entire secretome of whole genome sequenced invasive pneumococal strain TIGR4 and conducted an antibody inhibition assay with 62 monoclonal antibodies directed against the active sites of surface receptors, including all known receptors involved in the process of transmigration of leukocytes. Fishing with recombinant PSGL-1-fc was performed with protein-G beads in the isolated TIGR4 secretome. Western blotting of PSGL-1was performed with two different monoclonal antibodies. TIGR4 knock-out of ZmpC was generated with a Janus-type cassette. ZmpC presence in different *S. pneumoniae* serotypes was detected by PCR. ZmpC was isolated by FPLC chromatography; anion exchange and size exclusion chromatography. Static adhesion studies were performed with calcein-labeled neutrophils on immobilized p-selectin.

Results: The screening showed the presence of a component in the secretome of TIGR4 that could inhibit the binding of a monoclonal antibody directed against the active site of P-selectin Glycoprotein Ligand-1 (PSGL-1). PSGL-1 has been identified as the principal ligand for P-selectin. It is expressed on most leukocyte including neutrophils and it has been shown to be critical for leukocytes rolling in the recruitment of leukocytes to the site of infection. Purified TIGR-4 secretome could also inhibit static adhesion of neutrophils to P-selectin, indicating that there is a functional active inhibitor of PSGL-1 present. Additionally, fishing with recombinant human PSGL-1 resulted in degradation of the receptor, indicating the role of a specific protease. Furthermore, addition of EDTA (a divalent cation chelator) resulted in loss of receptor degradation. Purification of this inhibitor was done by FPLC chromatography resulted in the isolation of a 200kD inhibitor. The purified protein was analysed by mass-spectrometry and identified as Zinc metalloprotease C (ZmpC). ZmpC presence in *S. pneumoniae* serotypes correlates a 100% with the ability of cleaving PSGL-1, furthermore a TIGR4 ZmpC knock-out could not cleave PSGL-1 anymore.

Conclusion: *Streptococcus pneumoniae* is able to produce an inhibitor of PSGL-1, thereby block the transmigration process; leukocytes are unable to leave the blood stream, and therefore won't reach the side of infection, weakening the innate immune response.

O101

Interaction between *Streptococcus pneumoniae*, receptors and brain endothelial cells in an experimental meningitis model

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Introduction: *Streptococcus pneumoniae* (the pneumococcus) is a gram-positive human pathogen that causes life-threatening invasive diseases such as pneumonia and bacteremia with high morbidity and mortality throughout the world. Moreover, it is the most common cause of bacterial meningitis and *S. pneumoniae* is thought to invade the meninges via the bloodstream by crossing the endothelial cell layer of the blood brain barrier. Specific receptors expressed on the cell membrane of endothelial and epithelial cells mediate the adhesion and invasion of *S. pneumoniae* to human cells. The platelet-activating factor receptor (PAFR) and the laminin receptor (LR) were found to be involved in the interaction between *S. pneumoniae* and endothelial cells of the blood-brain barrier. The poly immunoglobulin receptor (PIGR) is a putative mediator of *S. pneumoniae* interactions with the lung epithelium. In this study, we addresses the question how *Streptococcus pneumoniae* crosses the blood brain barrier. In particular, we study whether the endothelium of the blood-brain barrier is the cerebral compartment where the receptors are expressed, what the distribution of the receptors in the brains is and whether this coincides with the pneumococcal association with the endothelium. Using a bacteremia derived meningitis mouse model, we

were able to visualize, localize and determine the cerebral vascular endothelium class with which the pneumococci are associated.

Methods: Balb/c mice were intravenously infected with FITC-labeled (10^8 CFU) of S. pneumoniae, serotype 4, strain TIGR4 and were sacrificed at various time points shortly after infection to mimic the early stages of meningitis. Immunofluorescence on slides of the mice was performed to detect S. pneumoniae, the endothelial cells and the receptors.

Results: Co-localization of *S. pneumoniae* within the vessels of the blood-brain barrier was found to occur at specific anatomical sites within the brain. The type of vessels to which the pneumococci were attached varied depending on the anatomical site Expression of various receptors seemed higher in the subarachnoid space under the meninges than in the cerebral cortex. In addition, receptor expression was associated in most cases with the endothelium especially in the subarachnoid space.

Conclusions: Our immunofluorescence analysis shows that *S. pneumoniae* is associated to both the macrovascular and microvascular endothelium depending on the anatomical site. Suggesting that CNS invasion, the key event for development of meningitis, may occur at numerous sites throughout the brain. Moreover, these studies provide precious insight in the expression of the receptors on the bloood-brain barrier endothelium and their possible role in the occurrence of meningitis. Ultimately, this work will lead to a better understanding of how pneumococci breach the blood brain barrier and cause meningitis.

O102

Identification of novel pneumococcal adherence factors by a combination of genome-wide approaches

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Introduction: *Streptococcus pneumoniae* is a common and important human pathogen associated with different types of infections, ranging from localized benign to severe systemic diseases. Infection of the human host by *S. pneumoniae* begins with colonization of the nasopharynx, which is mediated by the adherence of bacteria to the epithelial cells of the upper respiratory tract. Neither host nor bacterial factors required for adherence are fully characterized yet. To identify novel bacterial factors involved in adherence, we employed a combination of a genome-wide negative-selection screen and transcriptional profiling of multiple pneumococcal strains.

Methods: For the genomic screen, mariner transposon mutant libraries of *S. pneumoniae* R6 (non-encapsulated) or TIGR4 (serotype 4), containing ~15,000 and 3,500 mutants, respectively, were used in adherence assays with human pharyngeal epithelial Detroit 562 cells. After 2h adherence, chromosomal DNA was extracted from the adherent (cell-attached), non-adherent (planktonic), and total library fractions, and subsequently used to generate mutant-specific DNA probes. Mutants with decreased capacity to adhere to Detroit 562 cells were identified by next-generation sequencing using Tn-Seq. For transcriptional profiling, eight pneumococcal strains of different serotypes were allowed to adhere to Detroit 562 cells for 4h. Subsequently, both the cell-attached and planktonic bacteria were collected and suspended in RNAprotect. RNA was isolated and labeled according to standard protocols and hybridized to a custom-designed Nimblegen array representing the genomes of all strains used in the expression analyses. Probes that cross-hybridized with Detroit cDNA were excluded from the analysis.

Results: The genome-wide screens in the R6 and TIGR4 strains identified 79 and 56 genes as essential for adherence, respectively, among which genes coding for known adhesion factors, such as the *ami* locus, *adcC*, *cbpG*, *ppmA*, *srtA*, and the DHH family proteins. Interestingly, expression of most of these genes appeared to be highest in the planktonic fraction, suggesting they are priming for adherence. Expression of other genes known to be involved in adherence, such as *pspA*, cbpA, and the pilus islet, was found to be significantly upregulated in cell-attached bacteria in most strains. Furthermore, different sets of transporter genes were both found to be differentially expressed during adherence and identified in the screen, highlighting the importance of nutritional balance during this process. Finally, we identified several genes with an as yet unknown link to adherence, encoding mostly cell envelope located proteins, or genes annotated as hypothetical or of unknown function, which affected adherence to Detroit cells.

Conclusions: To further our understanding of the molecular aspects of pneumococcal adherence, we examined both gene expression and gene essentiality during *in vitro* adherence to Detroit 562 cells. Both genome-wide approaches confirmed the function of known adhesion factors, and identified various potential novel candidates. The specific role of identified genes in the adherence process is currently being investigated by various means.

O103

Membrane attack complex deposition on gram-positive bacteria

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Introduction: The complement system is essential in the host innate immune response against bacterial infection. This system fulfils important functions, including labelling of bacteria for phagocytosis or direct killing by assembly of the Membrane Attack Complex (MAC or C5b-9). During complement activation, C5b binds C6 and C7 exposing a hydrophobic site in C7 that allows initial binding of C5b67 to the bacterial membrane. Subsequent binding of C8 induces binding and polymerization of 10-16 C9 molecules to form the pore-forming structure known as the MAC. MAC formation on gram-positive bacteria is considered to be irrelevant since these bacteria are resistant against MAC-mediated lysis. Paradoxically, several gram-positive bacteria secrete proteins that specifically inhibit MAC formation and support bacterial survival *in vivo*. These findings suggest a role for the MAC in host defence against gram-positive bacteria and led us to investigate MAC deposition on these bacteria.

Methods: MAC deposition on bacterial surfaces was analyzed by flow cytometry using an antibody recognizing polymeric C9 or by immunoblotting. Confocal microscopy was used to localize the complex on the bacterial surface.

Results: Here we demonstrate that, upon incubation of bacteria with serum, the MAC is deposited on various gram-positive bacteria including Group A Streptococcus, *Bacillus subtilis*, Staphylococcus aureus, Group B Streptococcus, Lactococcus lactis, Staphylococcus epidermidis and *Streptococcus pneumoniae*. We observed a dose-dependent MAC deposition in serum, which could be inhibited by specific complement inhibitors or heat-inactivation of serum. In serum depleted from C6, C8 or C9 we could not detect MAC on the bacteria while deposition was restored by repletion with purified components. Moreover, we found that initial binding of the MAC to gram-positive bacteria is mediated by C5b67 while the complex is stabilized by incorporation of C8 and C9 molecules. Immunoblotting showed that bacterium-bound C9 is present in both monomeric and polymeric (SDS-stable) forms indicating that C5b-9 forms a ring-structure on gram-positive bacteria. Using confocal microscopy we showed that the MAC deposits on a specific location on the bacterium. This is in contrast to C3b deposition that occurs more random on the entire bacterial surface. On Group A Streptococcus, the MAC deposits specifically near the division septum whereas on *Bacillus subtilis* the complex is located at the poles.

Conclusions: Taken together, our data show complement-specific MAC deposition on gram-positive bacteria. Moreover, the MAC is deposited on a specific location on the bacterial surface. These results can provide a novel view on the role of MAC in host defence against gram-positive bacteria. Currently we are investigating the physiological role of MAC deposition. Furthermore, we aim to elucidate the molecular structure of bacterium-bound C5b-9 and identify the target of interaction on gram-positive bacteria.

O104

CXCL13 as a diagnostic marker for Lyme neuroborreliosis

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Apart from the skin, the nervous system is the most frequently affected organ in Lyme borreliosis. The main and most characteristic clinical symptom of Lyme neuroborreliosis (LNB) in Europe is Bannwarth's syndrome. It is characterised by intense, pseudoradicular pain and focal neurological deficits like for example paresis of the limbs or the facial nerve. The diagnosis can be easily made in such typical clinical cases, especially if accompanied by Erythema migrans. It can also be, however, a challenge in atypical cases, especially, if the laboratory tests are ambiguous.

The search for intrathecally produced, *Borrelia burgdorferi* -specific antibodies – as defined by the cerebrospinal fluid (CSF)-serum antibody index (AI) – can offer several pitfalls. On the one hand, a false-positive AI can be the result of an asymptomatic or subsided infection in the past. Furthermore, the intrathecal antibody production can remain for years even after successful treatment. On the other, a false-negative AI can be found in early cases of disease.

Both diagnostic gaps appear to be closed by a novel biomarker for LNB, the chemokine CXCL13. It is a B-cell attracting cytokine that is involved in the assembly of a B-cell enriched lymphocytic CSF pleocytosis as typically found in patients with acute LNB. CXCL13 can be found in high concentration in the CSF of these patients, even before the intrathecal production of *Borrelia burgdorferi*-specific antibodies has begun. In addition, the concentration of CXCL13 rapidly declines under antibiotic therapy and helps to discriminate a subsided from an active infection.

There are, however, some rare diseases of the central nervous system, that can lead to an increment of CXCL13 in the CSF and have to be considered as potential alternate diagnoses, as for example lymphoma or fungal infections. A prospective study, however, has shown that the sensitivity of CXCL13 is higher than the AI with an equal specificity. Therefore, CXCL13 is a useful and important additional marker in the diagnostic workup of patients with suspected acute LNB.

O105

Testing for Lyme disease: when and how?

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Diagnosing Lyme disease can be a challenging task for a clinician. Culture of *Borrelia. burgdorferi* is time-consuming and has a low sensitivity; therefore serology

is the mostly applied test. Serology however still has some disadvantages. Early in infection serology for many *B. burgdorferi* antigens can still be negative, while disease is already prominently present. Positive interpretation of immunoblots is often relying on presence of antibodies against several antigens. In that case it is possible that serology, which is high positive in the ELISA, can't be confirmed by an immunoblot. Later in infection antibodies against more antigens become prominent. In infections of a minimum of two months duration the serology is almost invariably positive for antibodies against a number of *B. burgdorferi* antigens.

First generation ELISA's used cultured whole cell sonicate *B. burgdorferi*, which often yielded many false positive results due to cross-reacting bacterial epitopes. Also, in culture many antigens that are important in mammalian infection are not expressed. Furthermore due to the many species in the *B. burgdorferi* sl complex that can cause Lyme disease the sensitivity of the ELISA was very much dependent on the strain used and the prevalence of *B. burgdorferi* sl species in that region.

Current modern screening ELISA's often use recombinant C6-peptide or VlsE as an antigen in ELISA, either alone, with whole cell sonicate, or combined with other recombinant proteins like outer surface protein C. It is however still important to either use a mix of, recombinant, proteins from several species or a highly conserved peptide to be able to screen for all species causingLyme infections.

For the immunoblot the line blots have the preference over native blots. *B. burgdorferi* immunoblots now use antigens that are important in mammalian infection, like VlsE, OspC, DbpA and Bmp. A mix of recombinant proteins of the most common species causing Lyme disease are used in several commercially available immunoblots.

After treatment, especially in early infection, serology for some antigens can return negative. Furthermore an IgM can sometimes be detected several years after infection. Also sometimes a positive IgM cannot properly seroconvert to IgG when treatment has been started early in infection. When a patient presents with a late manifestation of Lyme disease the IgM immunoblot is not of any value and might only cause more doubt. All these serologic oddities can lead to a wrong conclusion when the patients' history concerning Lyme disease is not taken into account by the interpreting clinician.

PCR has been applied widely for diagnosing Lyme disease. It can be helpful in early neuroborreliosis, although the sensitivity is low (~50%). PCR is often applied in Lyme arthritis on biopsies or punctures of the joints, where the sensitivity is reasonable (up to 80%). In Erythema Migrans and Acrodermatitis Chronica Atrophicans the results of the PCR have been very good. However, the results of the PCR's are never near a 100%, because of the low load of B. burgdorferi in the host in active infection.

Concluding, it is important in Lyme disease diagnostics to have the history and the complete clinical picture and duration of illness of the patient in interpreting the test results. Serology is still the test of choice, but PCR can sometimes aid in confirming the diagnosis.

O107

Comparison of the inter-laboratory performance of Lyme disease serology in the Netherlands by quality assessment

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Introduction: Serology is an important diagnostic tool in the diagnosis of Lyme disease. In most cases, a positive screening-assay result is confirmed by immunoblot assays. However, many different commercially serological assays are available. The comparability between these assays and the influence of different combinations is not clear, and may contribute to inter-laboratory variation. A serology quality assessment was therefore performed to investigate the influence of the different algorithms used in nine Dutch laboratories.

Methods: A total of 25 serum samples of Lyme patients with a well-defined diagnosis of erythema migrans (EM, n = 5), acrodermatitis chronicum atrophicans (ACA, n = 2), arthritis (n = 7) or neuroborreliosis (n = 1), and a serial diluted high positive sample (n = 5) and negative controls (n = 5) were analyzed by nine participating laboratories. These nine laboratories applied five different screening assays and four different confirmation assays in eight different combinations.

Results: Qualitative and quantitative values between laboratories using the same tests were within close range, indicating that inter-laboratory variation is low when the same assay is in use. However, qualitative results between different algorithms lead to some clear discrepancies and inter-laboratory variation. Discrepancies were mainly restricted to three proven Lyme cases with an early infection: two EM and one neuroborreliosis patient. All laboratories correctly identified *Borrelia*-specific antibodies in the two patients with ACA, and only minor variations in the arthritis patients were detected in the results between the participants. One laboratory reported *Borrelia*-specific antibodies in a patient diagnosed with non-Lyme arthritis. No positive results were reported in the three blood donors if the algorithm was applied.

Conclusions: Inter-laboratory variations in the serodiagnosis of Lyme-disease is mainly caused by the use

of different immunoassays and different algorithms. Differences seem mainly restricted to patients in the early phases of disease when antibodies are still present at a low level. However, this difference in test results between laboratories can undermine the trust of the public in the performance and quality of the diagnostic laboratories. Better awareness of inter-laboratory variation will hopefully lead to the further standardisation of Lyme serology between laboratories.

O108

Performance of five VlsE containing immunoassays for the diagnosis of Lyme borreliosis

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Objectives: The aim of the study was to assess sensitivity and specificity of five VlsE-containing assays for diagnosis of Lyme Borreliosis (LB).

Methods: During a six months period in 2010, clinical information on 558 patients, whose sera were sent to our laboratories for diagnosis of LB, was obtained by telephonic interview. All sera were screened by C6-EIA and if positive, tested by IgG and IgM immunoblot (Microgen). 477 of these sera (including all sera from patients with increased clinical suspicion for LB) were also tested in Diasorin, Serion, Medac and Enzygnost EIAs, as were 14 other sera from patients with disseminated LB, 92 sera from healthy controls and 94 control sera from patients with other diseases (infections with CMV, EBV, HIV, *M. pneumonae* and *T. pallidum*). All control sera, all clinical sera with a positive result in any EIA and 150 clinical sera which were negative in all EIAs were also tested in immunoblot. For sera from patients with atypical symptoms, an extended gold serologic standard was constructed, in which a serum was considered to be positive if a positive reaction was found in an immunoblot (IgG and/or IgM) or in at least 4/5 EIAs (IgG and/or IgM).

Results: We found sensitivities ranging between 57 and 90% for different EIAs in patients with early localized LB. For disseminated LB, the sensitivity of 4 EIAs was 100%; only one EIA missed 2/26 sera and therefore had a sensitivity of 93%. Specificities of EIAs ranged from 88 to 97% for healthy controls and 62 to 96% for other diseases. The sensitivity of the immunoblots in early localized LB was only 62%, whereas the specificity for healthy controls was 98%, but for patients with other infections 92%, due to a few false-positive IgM blots. Using the extended gold serologic standard for sera from patients with atypical symptoms, the sensitivities from EIAs ranged from 77 to 90% and specificities from 92 to 98% for detection of antibodies in these patients. No significant differences between the EIAs were found for diagnosis of early LB; Diasorin and Enzygnost showed the highest sensitivity and C6 and Medac performed marginally lower. Regarding specificity, C6 and Medac were superior to the other tests. Additional immunoblot tesing in clinical sera non-reactive in all EIAs gave no positive results.

Conclusion: Sensitivity of EIAs was very high for disseminated LB. In early localized LB, sensitivity of the assays was above results reported in other publications. Specificity of the assays differed, especially in sera from patients with other diseases. Although the value of positive Lyme serology in patients with atypical symptoms is debatable, generally a good concordance was found between the assays, although a number of discrepant results was also found.

O109

A generalised module for the selective extracellular accumulation of recombinant proteins

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It is widely accepted that laboratory strains of *Escherichia coli*, including those used for industrial production of proteins, do not secrete proteins to the extracellular milieu. Here, we report the development of a generalised module, based on the autotransporter secretion system, for the production of recombinant proteins in the extracellular compartment of *E. coli*.

We demonstrate that a wide variety of structurally diverse proteins can be secreted as soluble proteins when linked to the autotransporter module. Yields were comparable to those achieved with other secretion systems. The advantage of this module is that it relies on a relatively simple and easily manipulated secretion system, it exhibits no apparent limitation to the size of the secreted protein and can deliver proteins to the extracellular environment at levels of purity and yields sufficient for biotechnological applications.

O110

Structure and function o type Vc and type Ve autotransporters

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YadA is a trimeric autotransporter adhesin (TAA). Many members of the TAA family are important pathogenicity factors that mediate adhesion to host cells and tissues in such diverse diseases as diarrhea, urinary tract infections, or airway infections.

Solid-state magic-angle spinning (MAS) NMR spectroscopy has long been discussed as the emerging method of choice

for membrane protein structural biology. MAS NMR does not necessarily need highly and macroscopically ordered material. Moreover, solid-state NMR is a unique tool to study both dynamics and structure of proteins simultaneously at atomic resolution. I will present the first structure of a membrane protein, the transmembrane domain of the Yersinia Adhesin A (YadA), solved exclusively with solid-state MAS NMR data, using a single, uniformly 13C/15N labeled sample. In addition, NMR allowed us to acquire information on flexibility and other mechanistic details that cannot be transferred from the x-ray structure of a related protein, Hia. I will discuss implications for the autotranport process in general, and how the data fits to biochemical experiments on monomeric and other trimeric autotransporters.

O111

Versatile microbial Autotransporter platforms for the display and secretion of multiple heterologous proteins

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The self-sufficient Autotransporter (AT) pathway, ubiquitous in gram-negative bacteria, combines a relatively simple protein secretion mechanism with a high transport capacity. Capitalizing on its crystal structure, we have engineered the *Escherichia coli* AT Hemoglobin protease (Hbp) into versatile platforms for secretion and surface display of multiple heterologous proteins in one carrier molecule. As proof-of-concept, we show efficient secretion and high-density display of the sizeable Mycobacterium tuberculosis antigens ESAT6, Ag85B and Rv2660c in *E. coli*, both individually and simultaneously. Furthermore, we demonstrate stable multiple display of these antigens in an attenuated Salmonella typhimurium strain upon chromosomal integration, demonstrating the potential in the development of multivalent bacterial live vaccines.

O112

Specificity of target selection by the TpsB outer membrane transporters of co-existing Two Partner Secretion systems of the human pathogen *Neisseria meningitidis*

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Introduction: *Neisseria meningitidis* is a common cause of human diseases sepsis and meningitis and initiates its infection by colonizing the upper respiratory tract. N. meningitidis expresses multiple two partner secretion systems (TPS) to colonize and invade the epithelial cells. *In vitro*, the meningococcal TPS system 1 has been found in all strains tested to date, contributes to intracellular survival and escape from infected cells. TPS systems are composed of two components, a secreted exoprotein (TpsA) and a β barrel outer membrane protein (TpsB) that is thought to transport the exoprotein across the outer membrane. Recognition of the TpsB transporter requires the presence of a TPS domain at the N terminus of a TpsA. This recognition is thought to be system specific and restricted to cognate partners, often organized in an operon. However, three distinct TPS systems have been identified in meningococci with a different organization. Amongst them, systems 1 and 2 contain more than one TpsA and a single TpsB each, while system 3 contains a singular TpsA protein without a cognate TpsB translocator. **Results:** We have investigated the targeting and possible redundancy in the neisserial TpsBs. The system-2 TpsB showed a reduced specificity, which enables it to recognize and secrete the TPS domain of system 1 and 3, as well as more distantly related TPS domains of N. lactamica, but not of other bacterial species. We further determined the domains/parts of the neisserial TpsBs involved in substrate selection and subsequent recruitment for successful secretion. Our results provide insight in the target selection by a membrane-bound transporter protein involved in toxin related secretion.

O114

Multilevel approach indicates high clonal and functional diversity in Gouda cheese starter culture

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Gouda cheese starter cultures are complex, undefined blends of closely related strains of the species *Lactococcus lactis* and *Leuconostocs mesenteroides*. Starter cultures face several stresses like limited carbon and nitrogen source availability, osmotic stress due to brining, temperature fluctuations, pH changes and phage predation during cheese fermentation. The diversity of the microbial community in the starter culture is key for robustness of performance during the fermentation process. Unraveling the strain diversity in undefined dairy starter cultures requires a multilevel approach.

We have set out to explore the complexity of a BD type starter culture, including both *L. lactis* ssp *lactis* biovar. *diacetylactis* (D type) and *Leuconostocs mesenteroides* (B type), by combining culture based high resolution Amplified Fragment Length Polymorphism (AFLP) analysis,[1] plasmid profiling, phage predation tests, and metagenome sequencing. The dynamics in relative abundance of the identified genomic lineages during the course of cheese making provided indications for cooperation for proteolysis[2] and highlighted major population shifts after the brining step. Specific plasmid profiles appeared to enrich during the cheese ripening period. The diversity of phage sensitivity within the same genomic lineage explains robustness against clonal sweeps. Comparison of the diversity in genomic lineage type, plasmid profiles and phage sensitivity, reveals the complex and dynamic microbial community structure of the starter culture.

In general, starter cultures are preserved by back-slopping with restricted propagation regimes. Using molecular tools for tracking population dynamics at the level of strains, we studied the effect of different propagation regimes on starter functionality. These studies revealed massive changes in terms of population composition, acidification rate, and proteolysis. Interestingly, cheeses produced with the differently propagated cultures retained a similar genotype enrichment during the ripening period, indicating the robustness and the flexibility of the culture to environmental fluctuations.

References

1. Kütahya OE, Starrenburg MJC, Rademaker JLW, et al. Appl. Environ. Microbiol. 2011;77:5192-8.
2. Bachmann H, Molenaar D, Kleerebezem M, et al. The ISME Journal. 2011;5:1751-7362.

O115

Population dynamics in an undefined mixed starter culture – the role of bacteriophages

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Undefined mixed starter cultures used in cheese production can be regarded as complex ecosystems containing mixtures of strains belonging to the species of lactic acid bacteria such as: Lactococcus lactis and Leuconostoc mesenteroides. Usually, these mixed cultures also contain various lytic and temperate bacteriophages that co-exist with the bacterial strains. The constant-diversity dynamics model [1] predicts that predation of the microbial community by bacteriophages increases the diversity of the microbial community by preventing the domination of the culture by a single species. In this study, we set out to investigate the impact of the phage predation on the diversity and performance of an undefined dairy starter culture. Different genetic lineages, present in the model starter culture which is used in this study, are represented by a large collection of strains. Well characterized single colony isolates were used to create defined blends of strains (with and without the presence of bacteriophages) possessing specific characteristics (e.g. protease activity). The microbial population dynamics and the effect of phage predation in these blends was monitored during sequential propagation using real-time PCR amplification of unique genes associated with the different strains in the blend. The phage abundance was monitored by enumeration of plaques using phage sensitive indicator strains on agar plates. Finally, the ratio between protease positive and protease negative strains in the culture was determined using specific plating techniques. We found that prolonged propagation of the culture had an profound influence on its composition. The observed dynamics in relative abundance of bacteriophages in the culture demonstrates an equilibrium between phage sensitive and phage resistant variants in the culture during prolonged propagation.

References

1. Rodriguez-Valera F, et al.. Explaining microbial population genomics through phage predation. 2009;11:828-36.

O116

Immune evasion dynamics during *Staphylococcus aureus* biofilm growth

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Introduction: Planktonic bacteria growing in shakeflasks and sessile bacteria in biofilms behave very differently. The gene expression and production of secreted proteins is very different between these modes of growth. We study the dynamics in *S. aureus* biofilm growth and specifically the interaction of the biofilm with the human innate immune system. In our group we identified multiple immune evasion molecules produced by *S. aureus*. Although some *S. aureus* infections like sepsis could be regarded as planktonic, most infections like osteomyelitis, endocarditis, or prosthetic implant infections are biofilm-related (Hall-Stoodley and Stoodley, 2009). It is therefore highly relevant to study the immune evasive properties of *S. aureus* during the biofilm mode of growth.

Methods: To investigate the role of *S. aureus* immune evasion molecules in a biofilm context, we use both an adhesion-independent biofilm model and a continuous

biofilm flow cell setup. Using fluorescent protein reporters we study the expression dynamics of the immune modulating proteins expressed in *S. aureus* biofilms by flow cytometry and (confocal) fluorescence microscopy. Furthermore, by screening the proteins secreted by these biofilms we directly asses the production of known and novel immune modulating proteins.

By challenging the biofilm with compounds of the immune system while performing time-lapse microscopy we are able to study the interaction of the biofilm with these compounds in a direct and dynamic way.

Results and discussion: Immune evasion genes like *efb* and *ecb* are expressed strongly in planktonic cultures, whereas low expression was observed in the static biofilm assay. Generally, the total amount and number of proteins secreted during biofilm conditions is much lower compared to planktonic growth.

We focused on the expression of the Phenol Soluble Modulins (PSMs). These immune modulating peptides are highly expressed in planktonic cultures, and even higher during static biofilm growth. In flow cell biofilms their expression is switched on after the microcolonies become big enough. It was previously shown that the PSMs are capable of lysing neutrophils when reaching high enough concentrations (Wang et al., 2007). However, in conditions where serum is present, the PSMs are absorbed by serum lipoproteins. Inside and near biofilms the PSM concentration might be too high to be neutralized by the few serum lipoproteins that can penetrate this environment. Furthermore, *S. aureus* cells that are not producing PSMs will rapidly switch on their expression when phagocytosed by neutrophils. Due to the inactivation of PSMs in serum, it is likely that the PSMs are capable of lysing neutrophils only very near a biofilm, or in the intracellular environment.

The techniques we developed allow us to study expression of immune evasion genes inside a biofilm, and even after the bacteria are phagocytosed by neutrophils, providing insight in the immune evasion capabilities of *S. aureus* during infections.

References:

1. Hall-Stoodley L, Stoodley P. Cell Microbiol. 2009;11:1034-43.
2. Wang R, Braughton KR, Kretschmer D, et al. Nat Med. 2007;13:1510.

O117

Modulated lipooligosaccharide structure prevents Haemophilus influenzae from IgM-mediated complement killing during otitis media

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Non-typeable Haemophilus influenzae (NTHi) is a gram-negative, human-restricted pathogen. Although this bacterium typically colonizes the nasopharynx in the absence of clinical symptoms, it is also one of the major pathogens causing otitis media (OM). Complement represents an important aspect of the host defense against NTHi. In general, NTHi is efficiently killed by complement-mediated killing, however, various resistance mechanisms have evolved. The *in vivo* significance of such mechanisms and their role in the development of OM remains largely unknown.

To understand the role of complement resistance in OM, we analyzed complement resistance of NTHi isolates from the nasopharynx and the middle ear fluids of OM patients. We demonstrate that serum resistance was strongly increased for isolates from the middle ear, which correlated to decreased binding of IgM. GAF identified a crucial role for the R2866_0112 gene in serum resistance. Deletion of this gene altered the LOS composition of the bacterium, allowing for increased binding of IgM and complement-mediated lysis. In a novel mouse model of co-infection with influenza virus we demonstrate decreased virulence for the R2866_0112 deletion mutant.

These findings identify a novel gene that modulates the NTHi LOS structure to prevent recognition by IgM and activation of complement. Importantly, this mechanism plays a crucial role in the ability of NTHi to cause OM.

O118

Profiling the antibody response in acute and chronic patients from a recent Dutch Q fever outbreak by protein microarray

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Introduction: Q fever is a highly contagious zoonotic disease caused by *Coxiella burnetii*. The presentation of Q fever in humans is variable, from asymptomatic, acute and chronic disease to Q fever fatigue syndrome. It is diagnosed principally by serology and antibody patterns between phase I and II which differentiate between acute, convalescent and chronic Q fever. However, the current serological methods do not discriminate sufficiently or early enough between acute and chronic Q fever during the convalescent stage, making preventative treatment problematic.

Methods: We used a recently developed protein microarray to characterize the humoral immune response between acute and chronic Q fever patients from the Dutch outbreak to search for new serological markers to identify chronic Q fever. The array contained 93% of the proteome of Coxiella burnetii. The serological profile in samples from 13 acute and 12 clinically proven chronic patients were compared to control samples. The antibody kinetics and the influence of antibiotic treatment on the antibody response was studied in follow-up samples from 25 patients.
Results: Several serological markers were significantly present at higher levels in patients diagnosed with chronic Q fever compared to the acute patients. These detected markers may therefore be a better indicator for chronic disease than the level of the IgG phase 1 in the Immuno Fluorescence Assay. A decrease in antibody responses was observed in follow up serum samples after acute infection and during treatment of chronic Q fever. We observed possible reactivation in at least two chronic Q fever patients, indicated by an increase in the antibody responses to the identified markers after discontinuation of their antibiotic treatment.
Conclusion: While the Dutch Q fever outbreak has been challenging, the true challenge might still be ahead of us as we try to identify patients at risk, allowing us to prevent and treat chronic Q fever cases in the future. The serological markers we have identified may help us to identify patients at risk for the development chronic Q fever at an earlier stage than is now currently possible. They might also be used during follow up to monitor treatment response.

O119

Secretome phage display library, filling the gap between genomic data and functional studies

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Background: Secretome proteins contribute to pathogenic processes and are considered major virulence factors. Therefore identification and functional characterization of the secretome is essential in understanding pathogenesis, but classical methods are limited. Phage display technology is the process of expressing proteins fused to capsid proteins on the surface of a filamentous Ff-phage (viruses that specifically infect the gram negative bacterium *Escherichia coli* carrying F-pilli) and select the fraction of displayed proteins that exhibit a desired property. We tested the efficiency of the secretome phage display strategy to identify and functionally characterize the secretome of *Staphylococcus aureus*.
Results: The carboxy-terminal part of the phage capsid protein pIII was used to display *S. aureus* secretome proteins on filamentous fF-phages. A secretome phage display library was created using a phagemid vector devoided of any signal sequence or other membrane-targeting motif allowing the specific expression of secretome proteins. The *S. aureus* secretome phage library was selected for binding to various targets using a 96 well high throughput selection assay. After 3 selection rounds displayed proteins were identified by sequencing. With IgG as a target phages were selected displaying protein A, a well known IgG binding protein of *S. aureus*. Screening for interaction with an anti-CHIPS antibody and Von Willebrand factor selected phages displaying CHIPS protein and Von Willebrand Factor binding protein, respectively. Selection on C3b led to the simultaneous identification of phages displaying Efb and Sbi, which are both known to bind C3b. Phage display even allows use of whole cells as selection targets. When neutrophils were used this led to the selection of two secreted proteins, SelX and SSL6 after 4 selection rounds. SelX has recently been related to severe *S. aureus* infections. SSL6 belongs to a family of superantigen like proteins associated with immune evasion.
Conclusion: We describe an improved secretome phage display method resulting in a library with higher diversity and complexity compared to secretome phage display strategies previously published. For the first time we demonstrated that secretome phage display library is a very efficient technique to functionally characterize a bacterial secretome and especially to identify immune evasion proteins. Therefore, the strategy described here constitutes the ideal method to fill the gap between the increasing accumulation of genomic data and the lack of high throughput functional characterization methods.

O120

Induction of protective T-cell responses by *Aspergillus fumigatus* in humans

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Introduction: Aspergillus fumigatus is an ubiquitous fungus that can cause invasive infections in the immunocompromised host, but also in individuals with pre-existing lung injury. The protective immune response against invasive aspergillosis has been shown to rely on T-cell responses like the Th1 and Th17 response which are characterized by the cytokines IFN-γ, and IL-17 and IL-22 respectively. Studies in mice and humans indicated that several pattern recognition receptors (PRRs) like dectin-1, TLR-2 and TLR-4 play a major role in the induction of these essential immune responses. In this study we sought to

determine which pattern recognition receptors(PRRs) in humans play a crucial role in the induction or inhibition of protective T-cell responses by A. fumigatus.
Methods: From cultures of a clinical isolate of *A. fumigatus* (V05-27) conidia were harvested and used to grow hyphae. Both the conidia and hyphae were heat inactivated and subsequently used to stimulate human PBMCs. The PBMC were stimulated in presence or absence of specific inhibitors for dectin-1, TLR-2 and TLR-4, after 7 days cytokines IFN-γ, IL-22 and IL-17 were measured by ELISA.
Results: We found a redundant role for dectin-1 signalling, since blockade of dectin-1 or inhibition of its downstream signalling kinase SYK did not result in decreased cytokine production. Furthermore PBMCs from a dectin-1 deficient patient demonstrated normal capacity to induce these cytokines. Inhibition of TLR-4 and TLR-2 resulted in a differential outcome, inhibition of TLR-4 decreased IFN-γ, IL-22 and IL-17 responses whereas blockade of TLR-2 resulted in increased production of these cytokines.
Conclusion: We demonstrated, in contrast to earlier studies in mice, a redundant role for dectin-1 signalling in induction of protective T-cell cytokines like IFN-γ, IL-22 and IL-17 in humans. Blockade of TLR signalling demonstrated stimulatory a role for TLR-4, however TLR-2 inhibits induction of the studied T-cell cytokines which suggests an anti-inflammatory role for TLR-2 in the response against Aspergillus. Overall this study provides a new insight in the involvement of Dectin-1, TLR-2 and TLR-4 signalling in induction of protective immune responses against *A. fumigatus* in humans. Furthermore, these results may contribute in the development of new immunotherapies in the treatment of invasive aspergillosis.

O121

A general secretion signal for the type VII protein secretion pathway in pathogenic mycobacteria

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Pathogenic mycobacteria utilize specialized type VII protein secretion systems (T7SSs), designated ESX-1 through ESX-5, to secrete virulence factors. Although most T7SSs are involved in the secretion of small proteins belonging to the WXG100 protein family, the ESX-5 system is also responsible for secretion of numerous PE and PPE proteins. PE and PPE proteins are unique to mycobacteria, are heavily expanded in pathogenic species, and lack classical secretion signals. This study aimed at unraveling the signal(s) responsible for targeting PE and PPE proteins for ESX secretion.
The known ESX-5 substrates PE25/PPE41 form a dimer for which the structure has been elucidated. First, we determined whether these proteins also form a dimer upon secretion via the ESX-5 system. Pulldown experiments showed that these proteins are indeed secreted as a dimer. To identify sequences required for targeting of the PE25/PPE41 complex to ESX-5, systematic deletions in the regions encoding the N- and C-termini of these proteins were generated, and protein secretion was analyzed. These termini could not be elucidated in the structure and therefore probably form flexible loops that do not participate in dimer formation. As expected, all these mutants could still be precipitated in pulldown expriments, showing dimer formation. Strikingly, a small deletion in the unstructured C-terminus of PE25 completely abolished secretion of both PE25 and PPE41, indicating that this domain contains the secretion signal. Using a set of point mutations we showed that two conserved amino acids within this C-terminus are crucial for substrate recognition. To investigate if this signal also determines the T7SS specificity, we studied similar substrates that are secreted via another T7SS, < i*e.* ESX-1. Similar to the ESX-5 PE/PPE substrate, a small deletion in the C-terminus of an ESX-1-dependent PE protein was found to abrogate export. Intriguingly, replacement of the C-terminus of the ESX-1-dependent PE protein by the equivalent part of PE25 restored secretion, but did not alter the secretion route.
Together these data show that PE/PPE proteins are targeted for ESX secretion by a conserved signal in the C-terminus of the PE protein, but this signal does not determine the specificity of the system. Currently, we are producing chimeras of the ESX-1 and ESX-5-dependent PE proteins in order to unravel the system-specific requirements for secretion.

O122

Staphylococcus aureus secretes three Extracellular adherence proteins (Eap) that inhibit Neutrophil elastase

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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. *S. aureus* can survive

within the human body thanks to its numerous immune-evasion proteins that target critical steps in the innate immune system, our first line of defense against invading bacteria. Neutrophil elastase (NE) plays an important role in innate immunity, for it can kill bacteria both intracellularly (after phagocytosis) and extracellularly (within neutrophil extracellular traps; NETs). Surprisingly, no bacterial inhibitor of NE has been found to date.

In this study, we discovered three potent inhibitors of NE in *S. aureus*. The inhibitors of NE were identified by fractionating supernatant using different protein-purification techniques (ion exchange and size exclusion chromatography). The recovered fractions were tested in a functional NE assay. Subsequent mass-spectrometric analysis and recombinant expression identified Extracellular adhesion protein (Eap) as the NE inhibitor. In addition to Eap, *S. aureus* also encodes two Eap homologues named EapH1 and EapH2, of which the function is unknown. Here we show that EapH1 and EapH2 also potently inhibit NE, whereas other secreted immune-evasion proteins of *S. aureus* do not.

Eap can consist of 3-6 serially linked EAP domains of 11 kD. The number of domains differs among strains. Mutational analyses revealed that multiple EAP domains provide stronger inhibition of NE. In concordance, we find that EapH1 and EapH2 (which comprise only one domain each) are equally potent as a single domain of the *S. aureus* Mu50 Eap. Furthermore, the 5-domain-containing Eap of *S. aureus* Newman (65 kD) is more potent than the 4-domain-containing Eap of *S. aureus* Mu50 (52 kD). The mode of action is still unclear, but ELISA and Biacore data indicate that all three inhibitors bind to NE. In addition, the inhibitors are specific for NE, since they do not block Macrophage Elastase (ME or MMP12).

In conclusion, we show that (1) *S. aureus* secretes three proteins that inhibit NE, namely Eap, EapH1, and EapH2. Furthermore, (2) the more EAP domains these proteins contain, the better the NE inhibition is. Finally, (3) all three inhibitors bind to NE and (4) this inhbition is specific. Since NE is involved in many antimicrobial strategies of neutrophils, these inhibitors could be important for staphylococcal escape from immune defenses.

O123

Discovery of Schmallenberg virus

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In summer and autumn 2011, farmers and veterinarians in North Rhine-Westphalia (Germany), but also in the Netherlands, reported about mild clinical signs in dairy cattle including fever, reduction of milk production and diarrhea. Cattle in several farms showed a similar clinical picture, which disappeared after some days. Most cases in Germany were reported in September and October, and blood and swab samples taken from diseased cattle were tested by real-time RT-PCR for all common bovine viruses like bluetongue virus, epizootic hemorrhagic disease virus, pestiviruses, foot-and-mouth disease virus, bovine ephemeral fever virus or Rift Valley fever virus. However, all samples tested negative and none of the known pathogens could be connected to those cases.

Therefore, a metagenomic approach was chosen to analyze a pool of three selected samples from dairy cattle from a farm near the city of Schmallenberg which had fever and showed a decrease in milk production of more than 30%. The samples were analyzed following a protocol for RNA and DNA preparation and subsequent next generation sequencing in a *Genome Sequencer FLX* instrument (454/Roche) in November 2011. Using a newly developed software routine, all reads were compared to sequence databases, resulting in 7 reads showing a high homology to viruses of the genus *Orthobunyavirus*.

This sequence information was used to develop a specific real-time RT-System which allowed investigation of all collected blood samples from affected cattle farms. Subsequently, the first 5 positive farms could be detected, confirming the presence of Schmallenberg virus genome in the blood of several cattle. Subsequently, the virus could be isolated on cell culture and a first animal trial in calves was conducted. In addition, the full-length sequence was determined, and it could be shown that the new virus is most related to viruses of the so-called Simbu serogroup, especially to Shamonda virus.

After transferring the real-time RT-PCR to other laboratories in Europe, Schmallenberg virus could be also detected in samples from ruminants in the Netherlands, Belgium and UK. Since December 2011, Schmallenberg virus could be mainly found in brain samples of malformed lambs and calves showing the typical arthogryposis-hydranencephaly-syndrome.

With this presentation, the recent data and news about Schmallenberg virus will be provided and the use and power of metagenomics for diagnostics and pathogen discovery will be especially discussed.

O124

Longitudinal next generation 'deep' sequence analysis of dual/mixed HIV infected patients treated with Maraviroc demonstrates rapid selection for X4-predicted virus with extremely low FPR

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Background: Limited information is available on virological response after Maraviroc (MVC) treatment of patients with dual/mixed (D/M) virus. It has been shown that MVC can inhibit of a specific population of dual-tropic viruses that have a preference for CCR5 usage (dual-R5). We investigated the selective pressure of MVC on the viral population in four patients experiencing MVC treatment failure using next generation "deep"sequencing methods.

Methods: At baseline, four individuals harboured Dual/Mixed HIV populations according to the Monogram Trofile assay and/or "deep"sequencing. All patients received Maraviroc add-on therapy to investigate its additive value. Longitudinal 454 "deep"sequence analysis using triplicate HIV V3 RT-PCR was performed on plasma samples obtained over the following 6-26 days. A median of 12,000 sequence reads per sample were obtained. The geno2phenocoreceptor algorithm (g2p) was used for sequence interpretation.

Reults: Before MVC treatment, patients had a median total viral load of 4.7 log10 copies/ml; consisting of a median of 23% X4-virus (g2p FPR cut-off: 3.5). Upon MVC exposure, all four individuals showed decreases of the R5-viral load with a mean of 2 logs but the R5-population was replaced by X4 variants within 21 days. Remarkably, X4 variants with extremely low FPR values predominated after MVC – virus with FPR values of 1.3, 1.7. 1.8, and 1.1 accounted for > 90% of the circulating virus populations in these individuals respectively. In three of four patients, these X4 variants had expanded to fill the "space"left by the suppression of the R5 variants. This suggests that the virus setpoint remains similar regardless of whether virus producing cells express CCR5 and/or CXCR4. Population genetic estimates of viral fitness in the presence of MVC indicated a steep rise around a FPR value of 2, indicating that the selective advantage in the presence of MVC is the greatest for viruses with the lowest FPR values.

Conclusion: Longitudinal analysis of the independent CCR5 and CXCR4-using HIV population using next generation sequencing shows that MVC selects for viruses with an extremely low FPR. These data suggests that the antiviral activity of MVC may extend to a broader range of HIV variants than currently suspected.

O125

Evaluation of persistence of resistant variants with ultra-deep pyrosequencing in chronic hepatitis C patients treated with telaprevir

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Introduction: Worldwide, an estimated 170 million people are chronically infected with hepatitis C virus (HCV), which is a major cause of liver cirrhosis and hepatocellular carcinoma. HCV-related end-stage liver disease is now the main indication for liver transplantation in North America and Western Europe. The current standard of care, pegylated interferon-α-2a (Peg-IFN) and ribavirin (RBV) combination therapy, has a low sustained viral response (SVR) in patients infected with HCV genotype 1. Telaprevir, an orally bio-available NS3/4A protease inhibitor, has demonstrated potent antiviral activity in combination with Peg-IFN and RBV compared with Peg-IFN/RBV alone in HCV genotype 1 infected patients. In some patients who do not achieve an SVR, drug-resistant variants have been reported. A better understanding of the long-term persistence of these variants is needed. The aim of this study was to assess the prevalence of resistant variants in patients both before treatment with telaprevir and at long term follow-up by using the highly sensitive ultra-deep pyrosequencing (UDPS) technique.

Methods: Fourteen patients were recruited from 2 phase 1 clinical studies (VX101 and 103) of telaprevir. In these trials patients received either telaprevir monotherapy for 14 days or combination therapy with Peg-IFN. Previously well-described resistant variants at NS3 protease positions V36, T54, R155 and A156 were assessed at baseline and after a follow-up of 4±1.2 years by UDPS on the Roche 454 GS FLX platform. Resistant variants were tabulated at each time point and compared statistically between time points at each position using Fisher Exact tests, with Type I error controlled with the Bonferroni correction.

Results: Median number of sequence reads per position ranged from 4677-13326. Prevalence of variants associated with resistance to telaprevir at baseline was found to be very low. The highest baseline prevalence was observed in a patient carrying a T54A variant (0.54%). No resistant variants were detected in 9 of 14 patients at baseline as compared to 8 of 14 at long-term follow-up. In 13 of 14 patients, there was no indication of significant enrichment of resistant variants at the long-term follow-up time point relative to baseline, despite the presence of resistant variants in the clinical trial in all patients. The remaining patient had both V36M and T54S significantly enriched at

long-term follow-up (4.5% and 0.35%, respectively) relative to baseline, where neither variant was observed.
Conclusion: In patients treated for 14 days with telaprevir monotherapy or in combination with Peg-IFN, UDPS indicated that resistant variants do not typically persist long-term.

O126

HIV-1 Reverse Transcriptase drug resistance mutations M184V, M184I and M184T have a differential impact on entecavir incorporation and susceptibility

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Background: Entecavir is a potent antiviral drug for treatment of hepatitis B virus infections, but can also inhibit HIV-1. This was, among other experiments, demonstrated by selection of M184V or M184I in HIV-1 RT in the presence of entecavir, leading to reduced entecavir susceptibility. During antiviral therapy with other nucleoside analogues, M184V and M184I are the most frequently observed resistance associated changes at this codon, although other changes, like M184T, have been reported as well. We analyzed the effect of M184V, M184I and M184T on entecavir susceptibility and DNA synthesis.
Methods: Entecavir susceptibility for the different 184 variants (M/I/V/T) was analyzed in multiple cycle assays using MT2 cells or PBMC. Furthermore, we studied to what extent the 5'-triphosphate form of entecavir and its natural counterpart dGTP can act as substrates for the different 184 RT variants in the context of single nucleotide incorporations. In addition, we examined the processivity of the different 184 variants in absence of entecavir by analyzing multiple nucleotide incorporations under single-turnover conditions.
Results: The M184V and M184I, in comparison with wild type, gave rise to entecavir resistance (IC50 > 200 μM in MT2 and> 25 μM in PBMC). In contrast, the M184T was 83-fold hypersusceptible to entecavir in MT2 cells and 24-fold hypersusceptible in PBMC. The observed hypersusceptibility of M184T was specific for entecavir, since simultaneously performed control experiments revealed high-level resistance to 3TC (IC50 > 200 μM) and wild type susceptibilities to AZT and nevirapine. Remarkably, despite a severely compromised processivity, the M184T was able to incorporate entecavir monophosphate as well as its natural counterpart dGMP far more easily than M184V and M184I.
Conclusions: Increased entecavir incorporation efficiency explains why M184T is hypersusceptible to entecavir and is not selected during entecavir exposure. These results indicate that, although the V, I and T are all β-branched amino acids, they can have a large differential impact on entecavir incorporation and susceptibility.

O127

Comparison between Sanger cloning sequencing and ultra-deep sequencing techniques for the characterization of hepatitis C quasispecies

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Introduction: Hepatitis C virus (HCV) evolves as a population of closely related viruses with non-identical genomes, referred to as quasispecies. The quasispecies diversity may impact treatment outcome. Characterization of this genetic diversity with clonal Sanger sequencing has been limited, as this technique is labor intensive and the number of viral variants sequenced is relatively small. These limitations have been overcome with the development of ultra-deep sequencing (UDS) method that allows the detection of viral variants present with frequencies < 0.1%. The genetic diversity obtained by UDS is inflated without any error correction, and this process remains challenging to date. Nevertheless, UDS has the potency to replace clonal sequencing. However, the concordance between these two techniques has not been investigated in great detail. Therefore, we compared the inferred intra-host HCV variation between clonal Sanger sequencing and UDS.
Methods: HCV RNA was extracted from 15 chronically infected patients with either genotype 1a or 1b. Samples were collected at two time points, resulting in 30 clinical isolates. From these, the NS3 region was sequenced with both clonal Sanger sequencing and UDS using the Roche 454 GS FLX platform with the amplicon approach. Two amplicons with the size of 251 and 368 bp were generated, targeting the regions containing mutations associated with resistance to the new HCV protease inhibitor NS3. UDS data, i.e. reads, were processed with the QUASR pipeline and custom-made scripts to reduce technical errors. Reads were quality controlled, non-HCV sequences were removed by mapping, indels occurring after a homopolymeric region were corrected and reads not spanning the entire amplicon were discarded. For analytical purposes, identical viral variants were condensed to unique sequences. Major viral variants were defined here as unique sequences present in> 10% of the reads or clonal sequences. The inferred variants from both techniques were compared and analyzed with phylogenetic techniques and custom-made scripts, which returned the exact sequence matches between UDS and clonal sequencing.

Results: After error correction, in total 37.6% of the UDS data was lost. Discarded reads were either non-HCV sequences, short or low quality reads or reads that could not be matched to a sample. The median coverage was 5206 reads (range 445-18085) for UDS and the median number of clones was 84 (range 66-146) per sample. A median of 765 (range 38-2178) unique variants were inferred per sample with UDS and this was 37 (range 25-83) for clonal sequencing. In overall, major viral variants were inferred by both techniques. Nevertheless, the majority of the UDS variants, including a number of major viral variants were not detected by clonal sequencing. From two patients the inferred variants differed significantly between UDS and clonal sequencing.
Conclusion: We have demonstrated that, in general major HCV viral variants can be inferred with both clonal Sanger sequencing and UDS. However, pre-processing of UDS data is essential for a closer approximation of the true genetic diversity inferred with this technique. Ultimately, UDS detected higher genetic population diversity than Sanger cloning sequencing and may therefore be more suitable for the characterization of quasispecies.

O130

Recent trends in gonococcal resistance against third-generation cephalosporins and azithromycin

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Introduction: Decreasing antimicrobial susceptibility to third-generation cephalosporins and azithromycin among *Neisseria gonorrhoeae* (NG) has been described. An association between decreased susceptibility to cefotaxime and the presence of a *penA* mosaic gene was found in previous studies. Aim of the study was to monitor susceptibility trends and to investigate further dissemination of certain clonally related NG strains with decreased susceptibility to third-generation cephalosporins in Amsterdam..
Methods: The yearly number of NG isolates cultured in our laboratory varied between 865 and 1135. Resistance monitoring was done for cefotaxime from 2007-2011, for cefixime and ceftriaxone from 2010-2011 and for azithromycin from 2009-2011. Susceptibility testing of gonococcal isolates was done by Etests on GC-agar plates. The presence of a *penA* mosaic gene was determined with a conventional *penA* mosaic gene PCR. Clonality was assessed by both NG-MLVA and sequencing of the *penA* gene.
Results: The percentage of isolates with diminished susceptibility to cefotaxime (MIC ≥ 0.125, EUCAST breakpoint) was 7.9% in 2007 and increased to 12.6%, 11.8% and 13.7% in 2008 2010, respectively, whereas only 7.9% was found in 2011. Isolates with an MIC ≥ 0.125 against cefixime or ceftriaxone were hardly ever found (7 and 3 NG strains, respectively). Using an arbitrary breakpoint of ≥ 0.032, decreased susceptibility to cefixime and ceftriaxone fell from 17.6% to 11.2% and from 11.3% to 5.1%, respectively, between 2010 and 2011. From 2009 to 2011, the frequency of azithromycin-resistant strains varied between 7.5% and 9.9% without a trend. Among 74 NG isolates with an MIC> 0.125 against cefotaxime isolated from 2006-2008, 64% had a *penA* mosaic gene. All these isolates had PBP2 pattern XXXIV, which has been found earlier in San Francisco, Canada and Taiwan and were assigned to the same NG-MLVA cluster. In the period 2009-2010, 123 of 133 (93%) tested NG isolates with a cefotaxime MIC> 0.125 had a *penA* mosaic gene, as well as 30/33 isolates with a cefotaxime MIC ≤ 0.125, but a cefixime MIC of ≥ 0.064 or a ceftriaxone MIC ≥ 0.047. All *penA* mosaic positive NG isolates were assigned to the same NG-MLVA cluster, except for 10 isolates. Of 8 of these, as well as of 6 isolates belonging to the cluster, the *penA* gene was sequenced. These were again type XXXIV, with the exception of 4 isolates from 3 patients. These were assigned a different cluster containing isolates that had PBP2 pattern X, which was first identified in the WHO Western Pacific Region. Notably, two of these 3 patients were women, whereas the overwhelming majority of PBP2 type XXXIV isolates had been cultured from MSM.
Conclusions: Diminished susceptibility to cephalosporins among NG strains is widespread, although an unexplained decline seems to have occurred in 2011 in Amsterdam. One cluster with isolates with reduced cephalosporin susceptibility has been rapidly expanding, mainly among MSM, whereas a second type was also introduced. Resistance to azithomycin among NG strains is also common but did not increase in recent years.

O131

Improved diagnostics of bacterial vaginosis with molecular techniques

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Bacterial vaginosis (BV) is a disturbance of the vaginal microflora. BV can lead to pelvic inflammatory disease, ectopic pregnancy and premature birth. We evaluated a combination of PCR assay's and whole bacterial community analysis with the standard diagnostic algorithms. 160 women were evaluated according to Amsel criteria and gram stains from vaginal smears were made for Nugent scoring. Vaginal swabs were tested with PCR assays for Gardnerella vaginalis, Atopobium vaginae, BV associated bacterium type 2 (BVAB2)

and Megaspheara type 1 (MS1). Whole bacterial flora analysis was performed by 16S-rDNA PCR fragmentation (TRFLP).
Best overall performance was shown using a duplex real time PCR for BVAB2 and/or MS1 with a sensitivity of 90% and a specificity of 78% with respect to Amsel criteria. Using Nugent criteria as a standard, this duplex PCR has a sensitivity of 84% and specificity of 86%. From TRFLP results, the presence of predictive fragments of Prevotella, Aerococcus, Megaspheara, Mycoplasma, Peptostreptococcus, Leptotrichia, Eggerthella, Gardnerella, Atopobium and Dialister was most associated with BV positive samples. Cluster analysis of microbial profiles revealed clear differences between BV and non-BV and indicated possible intermediate or transition stages.
For molecular diagnostics a duplex PCR of Gardnerella and/or Atopobium can be used for initial screening confirmed by a BVAB2 specific PCR. A more effective alternative is a real time duplex PCR targeting BVAB2 and/or MS1. Microbial profiling supports most targets used in the PCR assays. Cluster analysis of microbial profiles can be used to interpret discordant validation results and possibly for diagnosis.
This abstract was presented in a oral presentation at the 19th biennial conference of the International Society for Sexually Transmitted Diseases Research (ISSTDR, Quebec 2011)

O132

Outcome of nosocomial *Clostridium difficile* infections; results of a multicenter cohort study

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Background: *Clostridium difficile* infections (CDI) are common in developed countries and mortality among patients with CDI is high. Studies concerning the mortality of CDI have mainly been conducted in outbreak situations or specific populations and the direct contribution of CDI to mortality is not well known. We aim to study the mortality due to CDI by comparing cases to matched controls and death certificates in an endemic situation.
Methods: Between 2006 and 2009, 13 Dutch hospitals participated in a cohort study. All hospitalized patients with diarrhoea and a positive assay for the toxin of *Clostridium difficile* were included. Within this cohort, 9 hospitals matched CDI patients to two control patients (one with diarrhoea and one without diarrhoea) during a minimum of 3 months. Matching was based on hospital, ward and time of CDI diagnosis. Survival status was obtained of all patients via the Dutch Civil Registration System, the cause of death was retrieved from the national registry of death certificates. Mortality was evaluated with a chi-square or t-test and Cox-regression analysis.
Results: We identified 1366 patients with CDI, corresponding with an incidence rate of 13 per 10,000 admissions. The cumulative all cause mortality was 13% after 30 days, 30% after 6 months and 37% after 1 year. 319 CDI patients were matched to 319 patients without diarrhoea and 236 patients with diarrhoea, who died in 5.4% and 8.6% respectively after 30 days. The absolute risk difference between cases and non-diarrhoeal controls was 9.5% and CDI patients had a 2.9 times higher chance to die within 30 days (Hazard Ratio 2.9, 95% CI 1.7-5.1). After adjusting for age, sex and underlying diseases CDI patients still had a Hazard ratio of 2.4. The cause of death, as registered in the death certificate, was related to CDI in 10% of the deaths (4% of all patients). Most patients that died because of CDI, died within 30 days (72%).
Conclusion: In an endemic situation, 13% of the patients die in the first 30 days after diagnosis, rising to 37% after 1 year. Our study used a unique way to study the effect of CDI on mortality risk, which showed that CDI increases the risk of death after 30 days between 8% (according to comparison of cases and controls) and 4% (according to death certificates).

O133

Prolonged bacterial culture to detect periprosthetic joint infection: how long is long enough?

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Introduction: Periprosthetic infection is probably the most feared complication of joint arthroplasty. The value of microbiological culture to diagnose periprosthetic infection is limited because standard methods may fail to detect biofilm-forming or fastidious bacteria. There is no clear agreement on the appropriate cultivation period. In this study we evaluate the duration of culture in relation to the clinical relevance of the isolates that were found.
Methods: From July 2009 to July 2011 periprosthetic specimens were extensively cultured on 3 different liquid and 5 different solid media for 21 days. Isolates were defined as clinically relevant if reporting the growth of the micro-organism to the clinician let to antibiotic therapy that was directed against this micro-organism. Time to final identification of organisms was monitored. If a series of periprosthetic specimens yielded multiple identical isolates, only the time to identification of the first isolate was included.
Results: A total of 269 periprosthetic specimens from 194 different patients yielded positive cultures. From these positive cultures, 320 unique isolates were identified and 217 of these isolates were considered clinically relevant. After 7 and 14 days, the detection rate via culture of the clinically relevant isolates was respectively 78 and 88%.

The median time to identification for the clinically relevant isolates was 3 days, median time to identification of the non-relevant isolates was 19 days. The micro-organisms that were detected most frequently were coagulase-negative staphylococci (30%), about half of these isolates were considered clinically relevant and the median time to identification was 6 days. The spectrum of species that were detected consisted further of 18% *Staphyloccus aureus* (100% relevant, median 2 days), 13% *Enterobacteriaceae* (88% relevant, median 3 days), 11% *Propionibacterium* species (19% relevant, median 22 days), other species were detected in frequencies below 6%.

Conclusions:

1. Prolonged bacterial culture is a necessary tool in the detection of periprostetic joint infections, because in the third week of culture still 12% of the clinically relevant isolates was identified.
2. With prolonged culture the chance of contamination grows. The median time to identification of the isolates that were considered contaminants was much longer than of the clinically relevant isolates.
3. Considering the results of this study, the transition point to culturing a vast majority of contaminants might be microbiological culture of 3 weeks, although evaluation of even longer culture can be necessary before this conclusion can be confirmed solidly.

O134

Evaluation of several biochemical and molecular techniques for identification of *Streptococcus pneumonia* and *Streptococcus pseudopneumoniae* and detection of these bacteria in respiratory samples.

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Introduction: Identification and detection of Mitis group streptococci has been hampered by the lack of sensitive and specific assays. Especially detection of *Streptococcus pneumoniae* in respiratory samples is susceptible to misidentification.

Methods: In this study we evaluated several biochemical and molecular assays for identification of *S. pneumoniae* and other Mitis group streptococci using a collection of 54 isolates obtained by routine culture of 53 respiratory specimens of patients with community-acquired pneumonia.

Results: The combined results of the biochemical and molecular assays indicated the presence of 23 S. pneumoniae, two *S. pseudopneumoniae* and 29 other Mitis group streptococci isolates. The tube bile solubility test that is considered as gold standard for identification of *S. pneumoniae* showed concordant results with optochin susceptibility testing (CO2 atmosphere) and a real-time multiplex PCR assay targeting the Spn9802 fragment and autolysin (lytA) gene. Optochin susceptibility testing upon incubation in O2 atmosphere, bile solubility testing by oxgall disk, matrix-assisted laser desorption ionization-time of flight mass spectrometry and sequence analysis of the tuf and rpoB genes resulted in several false positive, false negative or inconclusive results. The *S. pseudopneumoniae* isolates could only be identified by molecular assays and the multiplex real-time PCR assay was concluded to be most convenient for identification of *S. pneumoniae* and S. pseudopneumoniae. Using this method *S. pneumoniae* and *S. pseudopneumoniae* DNA could be detected in the respiratory samples from which they were isolated and in an additional 11 samples from which only other streptococci were isolated.

Conclusion: A multiplex PCR assay targeting the Spn9802 and lytA genes was shown to be a valuable tool for identification of *S. pneumoniae* and S. pseudopneumoniae and detection of these bacteria in respiratory specimens. Applying this method in a prospective analysis might elucidate the necessity of detecting S. pseudopneumoniae in a clinical microbiology laboratory.

O135

Routine identification of clinical isolates of anaerobic bacteria: matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry performs better than conventional identification methods

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Introduction: Routine identification of anaerobes by rapid and automated systems is often inaccurate or inconclusive, especially with uncommon or fastidious organisms. At our laboratory, MALDI-TOF mass spectrometry has already been introduced for identification of aerobic bacteria and yeasts. We compared MALDI-TOF MS with conventional biochemical methods to identify anaerobic bacteria in a routine clinical setting.

Methods: Clinical anaerobic bacteria, cultured consecutively between January 2010 and February 2011, were tested both by MALDI-TOF (Bruker Daltonik, Bremen, Germany) and conventional identification methods (API Rapid ID 32, bioMérieux, Marcy-l'Étoile, France). The results of MALDI-TOF MS and conventional identification were categorized as identical identification to the species level, identical identification to the genus level (if either or both techniques identified to the genus level only), discrepant results, or no reliable MALDI-TOF identification (score < 1.7). Discrepant results were further investigated by 16S rRNA gene sequencing.

Results: In total 296 anaerobic isolates were included in the survey. The most prevalent genera were: *Bacteroides* (25%), *Propionibacterium* (15%), *Prevotella* (13%), *Fusobacterium*, *Clostridium* and *Actinomyces* (8% each).

Of all isolates 225 (76%) were identified to the same genus or species by both methods, whereas 38 (13%) could not be identified by MALDI-TOF MS. Discrepant results were found in 33 (11%) of the isolates. With 16S rRNA gene sequencing as the gold standard 16 major errors were found using conventional methods while MALDI-TOF MS did not result in major errors. Minor errors were observed 8 and 2 times, respectively (Chi-square test, p = .009). Performance of MALDI-TOF MS did not vary between gram-positive and gram-negative bacteria. Results for *Bacteroides spp.*, *Clostridium spp.*, *Propionibacterium acnes*, *Finegoldia magna*, and *Prevotella spp.* were good; the identification results for *Fusobacterium spp.*, *Propionibacterium non-acnes spp.* and *Actinomyces spp.* still need improvement.
Conclusion: MALDI-TOF MS is superior to conventional techniques for the identification of anaerobic bacteria in a clinical setting. Further development of the database will be needed to optimize MALDI-TOF results.

O136

Empiric therapy for sepsis needs adaptation due to increased resistance rates

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Introduction: In 2011 the Dutch Society of Antibiotic Policy (SWAB) issued a new guideline on sepsis therapy. For hospital acquired (HA) sepsis of unknown origin it recommends combination therapies of piperacillin-tazobactam (PTZ), cefuroxim (CEF), cefotaxim or ceftriaxon (CFT) with aminoglycoside (AG) or ciprofloxacin (CIP), for community-acquired (CA) sepsis monotherapy with CEF or CFT or amoxi-clav (AMC) with AG, and for ESBL high risk patients carbapenems. In the present study, we investigated the distribution of species causing sepsis and to what extent the recommended empiric therapy covers sepsis caused by *Enterobacteriaceae* (ENT).
Materials and Methods: Antimicrobial Susceptibility Test (AST) results, first isolate per patient, from 2008 to 2010 were collected from the Infectious Diseases Surveillance and Information System for Antibiotic Resistance (ISIS-AR) at the RIVM, representing 27 laboratories covering 50% of the hospital beds. The distribution of species and the % I or R *Enterobacteriaceae* for the recommended regimens was calculated. *E. coli* (ECO) or *K. pneumoniae* (KPN) I or R to 3rd generation cephalosporins were defined ESBLs. Hospital acquired was defined as a blood culture (BC) obtained from an admitted patient and community acquired was defined as a BC obtained at the outpatient Dept. or emergency room.
Results: 35.113 (34% ENT) HA and 9.456 (36% ENT) CA isolates were included. The distribution of species was stable over the years, except for a significant decrease among HA and CA pneumococci and increase among HA *E. faecium*. From 2008 to 2010 (intermediate) resistance rates for HA ENT-BC isolates are significant increasing for all antibiotic therapies but remain below 5% except for CEF-CIP therapy (5.5%). These rates remained stable for the CA BC isolates but already since 2008 resistance rates for CEF and CFT monotherapy are above 10% and around 5% resp. Co-resistance rates among HA and CA ESBLs were, respectively: tobramycin (TOBR) 47% and 46%, gentamicin (GENT) 36% and 33%, for amikacin (AMIK) 9% and 5%, CIP 58% and 63%, carbapenems 0.2% and 0%.
Conclusion: From 2008 to 2010 the distribution of species causing sepsis was stable, except for a decrease among pneumococci and increase of HA *E. faecium*. Although the resistance rates among CA-ENT were stable, the increasing resistance rates among HA-ENT causing HA sepsis are worrisome. The recommendations of the SWAB guideline are greatly supported by our data, except for CEF-CIP therapy for HA sepsis and CEF or CFT monotherapy for CA sepsis since resistance rates in 2010 were above 5%, the consensus resistance rate above which a drug is no longer eligible for empiric sepsis therapy.

O137

Antimicrobial susceptibility testing in ninety minutes by bacterial cell count monitoring

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Introduction: The steadily rise of antimicrobial resistance has become a serious threat to global health. 'Superbugs"(harbouring for example NDM1 metallo-betalactamases) have emerged, and spread globally, which can hardly be treated with antibiotics considered safe enough for the patient. Paradoxically, research in new antibiotics has halted. Antimicrobial stewardship programs seem the only way to delay the rise of resistance and ascertain time to stimulate research in new drugs. These programs rely on quick access to susceptibility data. This study shows the proof-of-principle of bacterial cell count monitoring for rapid susceptibility testing.
Methods: Clinical isolates of Escherichia coli, *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains, identified by VITEK2® (bioMérieux) and Biflex® MALDI-TOF (Bruker Daltonics), were tested for amoxicillin / piperacillin and gentamicin by two commonly used methods (VITEK2® (VITEK) and Etest® (Etest)) and one reference method (broth-macrodilution (BMD)). Of each species a sensitive and a resistant strain was selected.

Susceptibility testing and interpretation of the results were performed using CLSI protocols and criteria. Antibiotic dilution series were produced in sixfold and included a positive (containing bacteria but no antibiotics) and negative (containing no bacteria and no antibiotics) control within each series. One series was processed directly by flow cytometry to ensure comparable bacterial cell counts between all broths at the start of the experiment. The remaining five series were incubated at 35C and processed by flow cytometry after respectively 60, 90, 120, 180, and 240 minutes of incubation. Processed series were discarded after analysis. Bacterial cell counts for broths within a series were compared to the positive control within that same series, in order to assure the same incubation duration. Differences in cell count increases (20, 40 or 60% less increase) as compared to the positive control were investigated for their ability to predict the MIC. For example, the broth with the lowest antibiotic concentration showing 40% less increase in bacterial cell count as compared to the positive control broth in the same series was considered to predict the MIC by flow cytometry.
Results: Susceptibility for *Escherichia coli* could reliably be predicted when cell count was 60% lower than the cell count of the positive control after 90 minutes. For *Staphylococcus aureus* a 60% lower cell count after 120 minutes predicted susceptibility and for *Pseudomonas aeruginosa* a 40% lower cell count after 120 minutes predicted susceptibility. No very major, major or minor errors were observed between bacterial cell count monitoring and VITEK, Etest or BMD. Time-to-result of bacterial cell count monitoring as compared to VITEK results was reduced by 74%, 76% and 83% for Escherichia coli, *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively.
Conclusion: Bacterial cell count monitoring can accurately predict susceptibility in 90 to 120 minutes and reduces time-to-result by at least 74% as compared to the fastest currently used method.

O138
CRISPR: a small RNA guided immune system

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An adaptive immune system in prokaryotes called CRISPR (clustered regularly interspaced short palindromic repeats) uses small guide RNAs to neutralize invading viruses and plasmids. In *Escherichia coli*, immunity depends on a ribonucleoprotein complex called Cascade. This protein complex recognizes double-stranded DNA from invaders by forming basepairs between the guide RNA and invader DNA. The recognition takes place without ATP consumption, indicating that invader continuous DNA surveillance takes place without major energy investments. Invaders can escape immunity by making point mutations in the guide recognition sequence. The structure of Cascade shows an unusual seahorse shape that undergoes conformational changes when it binds target DNA. This conformational rearrangement may serve as a signal to recruit the dedicated CRISPR-associated nuclease Cas3 that progressively degrades the target DNA in an ATP-dependent manner, neutralizing the virus infection.

References:

1. Brouns SJ, Jore MM, Lundgren M, et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. Science. 2008;15:321:960-4.
2. Jore MM, Lundgren M, van Duijn E, et al. Structural basis for CRISPR RNA-guided DNA recognition by Cascade. Nat Struct Mol Biol. 2011;18:529-36.
3. Semenova E, Jore MM, Datsenko KA, et al. Interference by clustered regularly interspaced short palindromic repeat (CRISPR) RNA is governed by a seed sequence. Proc Natl Acad Sci U S A. 2011;21:10098-103.
4. Wiedenheft B, Lander GC, Zhou K. et al. Structures of the RNA-guided surveillance complex from a bacterial immune system. Nature. 2011;21:486-9.

O139
crRNA maturation: a key event in the activation of the CRISPR/Cas immune system

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During their lifetime, all types of cellular life (Eukaryotes, Bacteria and Archaea) face the constant threat of parasite genome invaders. In Bacteria and Archaea, CRISPR (clustered, regularly interspaced short palindromic repeats)/Cas has recently emerged as an adaptive RNA-mediated defence mechanism against exogenous mobile genetic elements (phages, plasmids). The system also known as the prokaryotic RNA interference (RNAi) pathway is composed of CRISPR-associated (Cas) proteins and short RNA molecules (CRISPR RNAs: crRNAs). The crRNAs consist of unique repeat/spacer sequences and commonly guide the Cas protein(s) to cognate invading nucleic acids for their destruction. The CRISPR/Cas immune system has evolved into three main types with various subtypes and distinct mechanisms for activation of the system and silencing of the parasite genomes.
A key event in CRISPR activation is the maturation of crRNAs. My laboratory has recently focused on the system II and discovered a unique crRNA biogenesis pathway together with a novel biological function of CRISPR/Cas. Differential RNA sequencing of the model organism *S. pyogenes* led to the discovery of tracrRNA, a *trans*-encoded small RNA that displays complementarity to the CRISPR array at the level of the repeats. Using genetic and biochemical approaches, we demonstrated that tracrRNA base-pairs with each repeat sequence of the precursor

CRISPR molecule (pre-crRNA) to form a double-stranded RNA template that is cleaved by the host endoribonuclease III in the presence of the CRISPR-associated protein Cas9 (Csn1). We showed that the CRISPR/Cas machinery is constitutively activated to target and affect the maintenance of invading lysogenic phages that are known to carry crucial virulence genes. Each of the four components of the system, tracrRNA, pre-crRNA, RNase III and Cas9 are essential to protect *S. pyogenes* against prophage-derived DNA. As such, the system constitutes a bottleneck in the acquisition of new virulence genes by horizontal gene transfer. Furthermore, we provided evidence for expression and co-processing of tracrRNA and pre-crRNA in Type II systems of other bacterial species (*Listeria innocua, Neisseria meningitidis, Streptococcus mutans* and *Streptococcus thermophilus*) as well as co-evolution of tracrRNA anti-repeat and CRISPR repeat sequences.
To conclude, our study has revealed a novel pathway of small guide RNA maturation and the first example of a host factor (RNase III) required for bacterial RNA-mediated immunity against invaders. We also demonstrated a novel biological function of CRISPR/Cas in streptococci, i.e. limitation of the toxigenic conversion of streptococcal isolates. Thus, our work further highlights the remarkable diversity and complexity of the RNA-guided mechanisms of the CRISPR/Cas systems. With this regard, novel findings on the evolutionary aspects of the system II together with a comparative analysis with the systems I and III will also be discussed.

O140

Transcription of *Campylobacter jejuni* CRISPR RNAs is initiated from a sigma70 promoter located in each separate CRISPR repeat

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Background and aims: The **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats (CRISPR repeats), are found across many different bacterial and archeal species, and are thought to represent a prokaryotic form of an acquired immune system. The CRISPR system mediate resistance to bacteriophages and other forms of foreign DNA, with the mature CRISPR RNAs (cRNAs) molecules targetting the incoming foreign DNA for degradation. These mature cRNAs are commonly formed from a single precursor RNA, either via the activity of the CRISPR-associated (CAS) genes alone, or with the assistance of RNase III, which cleaves a 24 nt double-stranded RNA section formed by binding of a anti-CRISPR RNA (tracrRNA). Bioinformatic analysis suggested the latter mechanism to be present in *Campylobacter jejuni*, and in this study we have investigated transcription of the CRISPR region in *C. jejuni*.
Results: Differential RNA-seq analysis of the *C. jejuni* NCTC 11168 transcriptome demonstrated high-level transcription of the CRISPR array (located between Cj1519 and Cj1521c). Both dRNA-seq and 5' RACE analysis of transcription start sites in the CRISPR region demonstrated that transcription initiation occured from sigma70- promoters located at the 3' end of each CRISPR repeat, rather than from a single promoter upstream of the CRISPR array. This leads to multiple precursor RNAs each containing multiple repeat and spacer sequences. A non-coding RNA transcribed downstream of the CRISPR array has all the features of a tracrRNA, as it has 24 nt complementarity to the spacer/repeat sequences covering the 3' end of the mature cRNAs. Finally, bioinformatic analysis of CRISPR arrays predicted that similar transcriptional arrays may be present in species with this specific subtype of CRISPR-Cas (reduced Type II) throughout the bacterial kingdom.
Conclusion: Bacteria like *C. jejuni* have a uniquely organised, transcriptionally active CRISPR-system which may mediate resistance to foreign DNA, an important feature for a naturally competent organism.

O141

CRISPR-Cas system in the genomes of *Campylobacter fetus* subspecies

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Introduction: *Campylobacter fetus* (*C. fetus*), a microaerophilic spiral gram-negative bacterium, has been recognized as an important pathogen in both animals and humans. *C. fetus* subspecies are closely related on the genomic level, but infect hosts and niches with distinct preferences. *C. fetus fetus* (Cff) has been isolated from a variety of sites in different hosts. *C. fetus venerealis* (Cfv) is restricted to the genital tract of both male and female cattle and is the causative agent of the
reproductive disease Bovine Genital Campylobacteriosis. *C. fetus testudinum* ssp. nov. (Cft) has been isolated from reptiles and humans with underlying illness. In this study whole genome sequences were analyzed for the presence of regularly interspaced and short palindromic repeats (CRISPR) and adjacent associated cas sequences.

Methods: Whole genome sequences were obtained using Roche 454 sequencing of Cff strain 04/554, Cfv strain 97/608, and Cft strains SP3 and 03-427. A scaffold genome sequence of each strain was obtained, closed and annotated against the current genome annotation for Cff strain 82-40.
Results: All sequenced *C. fetus* strains contained clustered CRISPRs immediately downstream of the leucine encoding t-RNA. The Cff genome contained one set of six CRISPRs, Cfv contained one set of 19 CRISPRs, and Cft strains SP3 and 03-427 both contained three sets of 20/22/20 and 24/27/27 CRISPRs, respectively. Only in both Cft strains six adjacent cas genes were identified. In the CRISPR region of Cff a putative insertion element and multiple phage-like sequences were present; a phage-like sequence is also present in Cft strain SP3 but not in strain 03-427. In Cff and Cfv, the presence of phage-like repeat sequences suggests that recombination resulted in the loss of cas genes. In contrast to the other strains, Cfv strain 97/608 contains multiple inserted elements and phage like insertions outside of the CRISPR region.
Conclusion: In *C. fetus* the CRISPR locus contains multiple sets of repeats flanked by phagerelated genes that provide a hot spot for recombination. In Cff and Cfv this resulted in the loss of cas genes, and thereby inactivation of the CRISPR-Cas system. This might be associated with the presence of multiple inserted elements and integrated phages in different *C. fetus* strains.

O143

Post-genomic approaches to enhance intestinal robustness of probiotics

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Bacteria can adapt rapidly to changing conditions in their environment, using gene regulatory programmes that activate and/or repress functions involved in stress response and adaptation. This process is an important determinant for the robustness of performance of industrially applied Lactic Acid Bacteria (LAB). Moreover, probiotic LAB are expected to actively interact with the host intestinal tissues to provide a health benefit to the consumer, implying that these LAB have to withstand environmental challenges encountered during gastrointestinal transit.
Using *Lactobacillus plantarum* WCFS1 as a model for probiotic lactobacilli, this presentation will focus on the adaptation of this bacterium to the intestinal environment, identifying several biomarkers for gastrointestinal persistence. In addition, the use of differential fermentation conditions to pre-adapt these bacteria to increase their tolerance to intestinal conditions will be illustrated. The relevance of these approaches, is highlighted by transcriptome-trait matching that enabled the identification of several marker-genes that are contributing to GI-tract tolerance. Finally, current intestinal adaptive evolution strategies that can drastically extend the persistence of *L. plantarum* WCFS1 in the mouse GI-tract will be discussed.
Taken together this presentation will focus on the post-genomic unraveling of microbial adaptation to intestinal conditions and how such knowledge can be harnessed to enhance probiotic culture *in situ* delivery.

O144

Bacterial spore stress resistance; a proteomics quest for bio-markers

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Bacillus subtilis is an aerobically growing gram-positive spore forming bacterium. It Is generally used as the model organism for this class of bacteria. Bacterial spores are ubiquitous in the environment and cause problems in health care, food safety and food spoilage due to their high resistance to thermal stress, antimicrobial compounds of use in medicine and food preservation techniques. Upon persistence in foods spores may untimely germinate and give rise to vegetative cells that spoil foods and may, e.g. in the case of Bacillus cereus, also produce toxins provoking disease. Much research is geared towards the identification of strategies able to inactivate the spore germination capability or prevent successful spore outgrowth to the stage where vegetative cell division commences. Often harsh treatments are applied to ensure efficacy. However to come to more knowledge-based targeted approaches where microbial stability and safety are optimized versus food product quality and production costs, insight in spore structure and derived from that spore environmental persistence is needed.
Here we will discuss our proteomics approach to analyze the bacterial spore coat, considered key to stress resistance as coatless spores are vulnerable to many antimicrobial compounds as well as display increased heat sensitivity. Thus we set out to characterize the spore coat of a *B. subtilis* lab-strain and a food isolate using an innovative mass spectrometry technique. Previously, 70 proteins have been assigned to the spore coat of *Bacillus subtilis* using SDS-PAGE, 2-DE gel approaches, protein localization studies and genome-wide transcriptome studies. We now present a ''gel-free'' protocol for direct coat proteomics of *B. subtilis*[1] that is also capable of comprehensive B. cereus

ATCC 14579 spore coat protein and exosporium analysis addressing their insoluble fraction. Using LC-MS/MS We identified 74 proteins from the insoluble fraction of B. cereus ATCC 14579 spore coat and exosporium of which only 24 are previously assigned spore coat and exosporium proteins. Identification of spore coat proteins from a *B. subtilis* food-spoilage isolate corroborated the generic and 'applied' use of our protocol. Importantly, heterogeneity is commonly observed when analyzing germination and outgrowth under conditions representative for practical situations2. To analyze phenotypic heterogeneity of the spore coat and exosporium composition fluorescent reporter proteins will have to be generated and spore populations analyzed using single spore (live) imaging tools. Together the data should allow for the identification of suitable protein targets for the development of specific and sensitive spore detection and /or purification systems from food stuff or patient material. The protocol is currently extended to study cross-linking among the spore coat and exosporium proteins as well as their relative quantification.

References:

1. Abhyankar W, Ter Beek A, Dekker H, et al. Gel-free proteomic identification of the *Bacillus subtilis* insoluble spore coat fraction. Proteomics 2011;11:4541-50.
2. Ter Beek A, Hornstra LM, Pandey R, et al. Models of the behaviour of (thermally stressed) microbial spores in foods; tools to study mechanisms of damage and repair. Food Microbiology. 2011; 28:678-84.

P001

Accreditatie van nascholing in beeld

P002

Respiratory syncytial virus infection augments NOD2 signaling in an IFN-β dependent manner in human primary cells

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Respiratory Syncytial Virus (RSV) enters the human body through the nasopharynx, where it infects epithelial cells, leading to the recruitment of innate immune cells to the site of infection. The nasopharynx is colonized with gram-positive and gram-negative bacteria and previous studies have shown that their microbial products are translocated across the mucosa. Although the effects of bacterial ligands on the innate immune system are well-studied, less is known about their effect on the innate response during infection with RSV. To increase our understanding of these complex interactions, this study investigates how bacterial components modulate the pro-inflammatory response to RSV infection in human cells.

Human PBMCs from five healthy volunteers and five Crohn's disease patients homozygous for the 3020insC mutation to their NOD2 gene were stimulated for 24hrs with RSV A2, MDP and purified PRR ligands. Subsequently TNF-α was measured using an ELISA or real-time quantitative PCRs were performed.

Although most bacterial ligands did not show an increase in pro-inflammatory cytokines during an RSV infection, muramyl dipeptide (MDP) showed a synergistic increase in TNF-α and IL-1β. The nucleotide-binding oligomerization domain containing 2 (NOD2) receptor recognizes MDP, therefore PBMC from Crohn's disease patients homozygous for the 3020insC mutation in the NOD2 gene were used. PBMC from these patients did not show a synergistic response to stimulation with RSV and MDP, suggesting that NOD2 is essential for the observed synergy. Further experiments, aimed at identifying the viral ligand, suggested that dsRNA played an essential role. Stimulation with RSV or Poly(I:C) induced IFN-β expression, which resulted in an increased expression of the viral receptors TLR3 and RIG-I, as well as in NOD2. In this system, IFN-β induction by viral dsRNA is an essential first step in the increased pro-inflammatory response to MDP. We speculate that the enhanced pro-inflammatory response to bacterial ligands following RSV infection may be an important factor in determining the outcome of the severity of disease.

P003

Fast detection of *Bacillus anthracis* spores with MALDI-TOF-MS

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The first step to control a biological crisis is to ascertain that a biological threat is a real threat or a hoax. Fast information about the incident is crucial to reduce panic in case of a hoax or take countermeasures in case of a real threat. A serious biological threat is *Bacillus anthracis* in 'powder letters'. Currently detection of *B. anthracis* spores is based on PCR amplification and antibody-based techniques. To support these commonly used techniques a MALDI-TOF-MS test was developed. In this pilot study we show that MALDI-TOF-MS has potential to identify *B. anthracis* spores swiftly and accurately.

MALDI-TOF-MS is a rapid method able to identify bacteria. MALDI-TOF-MS emerges as a new diagnostic tool in established laboratories. The aim of our study was to develop an application able to identify swiftly realistic amounts of *B. anthracis* spores (< 1 mg). Therefore, three different sample preparation methods with three

different concentrations were tested with *Bacillus atrophaeus* (*globigii*) and *Bacillus thuringiensis* spores. With the preferred method MS-spectra were generated from *B. anthracis* spores and five potential hoax materials. From all obtained MS-spectra a small library was generated containing MS-spectra from *B. atrophaeus* (*globigii*), *B. thuringiensis*, and *B. anthracis*.

Next, 12 samples were tested. Interpretation of the MS-spectra were successful; all four *B. anthracis* positive samples were detected. Next, samples containing a hoax material, a simulant or a combination of hoax material, simulant or *B. anthracis* spores were identified correctly. In the 12 tested samples no false positive or false negative detections were made.

In conclusion; In spite of that we only tested a small number of strains this pilot study showed that the proposed fast MALDI-TOF-MS procedure is an easy method with high potential to identify *B. anthracis* spores swiftly and accurately.

P004

Phylogenetic analysis of the complete mitochondrial genome of *Madurella mycetomatis* confirms its taxonomic position within the order Sordariales

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Introduction: *Madurella mycetomatis* is the most common cause of human eumycetoma. The genus *Madurella* has been characterized by overall sterility on mycological media. Due to this sterility and the absence of other reliable morphological and ultrastructural characters, the taxonomic classification of *Madurella* has long been a challenge. Mitochondria are of monophyletic origin and mitochondrial genomes have been proven to be useful in phylogenetic analyses.

Results: the first complete mitochondrial DNA genome of a mycetoma-causative agent was sequenced using 454 sequencing. The mitochondrial genome of *M. mycetomatis* is a circular DNA molecule with a size of 45,590 bp, encoding for the small and the large subunit rRNAs, 27 tRNAs, 11 genes encoding subunits of respiratory chain complexes, 2 ATP synthase subunits, 5 hypothetical proteins, 6 intronic proteins including the ribosomal protein rps3. In phylogenetic analyses using amino acid sequences of the proteins involved in respiratory chain complexes and the 2 ATP synthases it appeared that *M. mycetomatis* clustered together with members of the order Sordariales and that it was most closely related to *Chaetomium thermophilum*. Analyses of the gene order showed that within the order Sordariales a similar gene order is found. Furthermore also the tRNA order seemed mostly conserved.

Conclusion: Phylogenetic analyses of fungal mitochondrial genomes confirmed that *M. mycetomatis* belongs to the order of Sordariales and that it was most closely related to *C. thermophilum*, with which it also shared both a comparable gene and tRNA order.

P006

In vitro antifungal activity of isavuconazole to *Madurella mycetomatis*, the causative agent of black-grain mycetoma

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Introduction: *Madurella mycetomatis* is the most prevalent causative agent of eumycetoma. This chronic, granulomatous infection is frequently found in the lower extremity, initially as a small subcutaneous nodule, which slowly progresses into swollen lesions and sinuses that discharge black grains. To date, treatment of eumycetoma in endemic areas still consists of a combination of extensive surgery or amputation of the infected tissue or limb and prolonged antifungal therapy with ketoconazole or itraconazole. Although successful cases have been reported, eumycetoma is associated with high recurrence rates, even after amputation of the affected limb.

Previous *in vitro* studies have shown that *M. mycetomatis* is susceptible to several azoles. Therefore, the antifungal activity to *M. mycetomatis* of the new azole isavuconazole (BAL4815) has also been explored. This second generation triazole, currently studied in Phase III trials, is a broad-spectrum antifungal agent shown to have good *in vitro* antifungal activity to several other causative agents of black-grain mycetoma.

Methods: In this study, the susceptibility of 22 *M. mycetomatis* isolates (obtained from 21 patients in the Mycetoma Research Centre, University of Khartoum, Sudan in 1999 and 2000) to isavuconazole was determined. A standardized hyphal suspension of *M. mycetomatis* was prepared in RPMI and exposed to 0.031-16 µg/ml isavuconazole, ketoconazole or itraconazole for 7 days at 37°C. To facilitate endpoint reading 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) was added.

Results: MICs ranged from 0.031-1 µg/ml, from ≤ 0.016-0.25 µg/ml, and from ≤ 0.016-0.125 µg/ml for ketoconazole, itraconazole, and isavuconazole, respectively. The MIC_{90} of isavuconazole was 0.063 µg/ml, which appeared to be lower to the MIC_{90} of ketoconazole (0.25 µg/ml) and itraconazole (0.125 µg/ml).

Conclusion: *In vitro, M. mycetomatis* appears to be highly susceptible to isavuconazole. Pharmacokinetic data show that the water-soluble prodrug isavuconazonium (BAL8557) is rapidly converted into the active compound (BAL4815), which has a longer half-life than other currently available triazoles. Further, it has extensive tissue distribution, high plasma binding capacity, and its toxicity is lower to that of ketoconazole and itraconazole. These properties make isavuconazole a promising antifungal agent in the treatment of mycetoma caused by *M. mycetomatis*.

P007

TEMPOtest-QC – An EU funded project to promote the development and use of microbiological point-of-care testing technologies in Europe

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Introduction: TEMPOtest-QC is an FP7-funded European project designed to help promote the development and use of rapid / Point-of-Care bacterial and fungal diagnostic technologies throughout Europe.
Methods: The project involves the development of various online "tools"and services, including: i) the development of a Europe-wide questionnaire collecting the views of diagnostic kit technology developers, general practitioners, medical microbiologists, and members of the general public (relating to perceived obstacles and/or benefits to the development and implementation of bacterial and fungal rapid / Point-of-Care testing technologies); ii) the creation of well characterized bacterial isolate and specimen biobanks (for use in validation studies by technology developers and in conjunction with the TEMPOtest-QC consortium); iii) a comparison tool to allow simple statistical analysis to be performed using kit test performance results, and the ability to compare these parameters to previously published diagnostic kit data; iv) a searchable database of antimicrobial resistance genes, which will be updated with mobile elements, virulence genes, and bacterial identification markers; and v) a list of useful links, including the names, websites and email addresses of companies / websites currently working in this field.
Results: Further details can be found on the website 'www.TEMPOtest-QC.eu'.
Conclusion: The project will initially run until 2013, and information / feedback from large industrial concerns, small and medium enterprises, and university Dept.s is encouraged.

P009

No novel coronaviruses identified in a large collection of human nasopharyngeal specimens using family-wide CODEHOP-based primers

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Introduction: A significant proportion of acute respiratory tract illnesses is caused by a wide variety of virus species, and those yet-to-be identified might be responsible for numerous disease cases with unknown etiology. The family Coronaviridae includes four circulating human coronaviruses (HCoV), HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1, as well as SARS-CoV, which was responsible for a contained zoonotic outbreak. To facilitate the detection of novel coronaviruses, we developed a modified CODEHOP protocol to design primers that recognize distantly related corona- and toroviruses (CT12-mCODEHOP) in a one-step reverse-transcriptase (RT)-PCR assay.
Methods: In this study the CT12-mCODEHOP one-step RT-PCR assay was used to screen 1800 nasopharyngeal swab samples obtained from adult outpatients with respiratory illness and asymptomatic matched controls over three consecutive winter seasons during the period 2007-2010. Thousand and sixty-four samples were collected from symptomatic adults at their first visit to the general practitioner (V1 samples), 484 were follow-up samples (V2 samples) obtained 4 weeks later, and 252 specimens were from subjects without respiratory disease symptoms. According to a multiplex real-time PCR, 79 of these samples were positive for HCoV.
Results: The CT12-mCODEHOP assay detected 36.7% (29/79) of the HCoV-positive samples, including HCoV-229E (1 specimen), HCoV-NL63 (9), and HCoV-OC43 (19). These specimens had relatively high viral loads corresponding to a median (interquartile range) cycle threshold value of 23.7 (21.2-26.4). Additionally, three specimens tested positive for HCoV-HKU1, which was not targeted by the real-time RT-PCR assay. No nonspecific amplification signals were observed.
Conclusion: The conducted study validates the CT12-mCODEHOP-based assay for selective identification of viruses of the family Coronaviridae. The exclusive detection of four known human coronavirus species by the broadly-reactive CoV/ToV assay in a large collection of

human respiratory specimens indicates that these viruses may account for the entire diversity of respiratory coronaviruses circulating in the human population.

P010

Detection of *Staphylococcus aureus* RNA improves detection from whole blood and is a potential marker to measure pathogen viability

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Blood stream infections are characterized by high morbidity and mortality. Fast and accurate identification of the pathogen causing disease is therefore important and can result in improved therapy. The time-consuming blood culture technique is still the gold standard to identify blood stream infections. Sensitive real-time PCR assays can improve time-to-result but are mostly developed for identification of DNA.

This study aims to investigate the value of RNA detection in addition to DNA from *S. aureus* spiked whole blood samples to increase sensitivity of molecular diagnostic assays. Additionally the value of RNA detection in relation to pathogen viability is investigated.

The detection limit of the assay was determined by spiking 1 ml whole blood samples with serial dilutions of log phase S.aureus bacteria. Samples were analyzed by performing real-time PCRs based on the *tuf* gene (singlecopy gene) and *16S rDNA* (multicopy gene).

Detection of both RNA and DNA did improve the detection limit of the tuf PCR assay. It resulted in lower Cp values and a yield of profit of at least 1 log compared to the DNA only samples. The samples containing low bacterial loads (10 CFU/ml) always showed a real-time PCR signal when RNA was additionally detected. RNA also seems to be a good marker for viability. With the tuf gene based test DNA was detected up to 72 hours after treatment with floxapen, whereas RNA was only present up to 48 hours. Using the 16S rRNA assay both DNA and RNA were detected up to 72 hours after treatment.

In conclusion, the detection limit as well as hitrate of the real-time PCR assay were improved by detection of both RNA and DNA. In addition, mRNA (tuf gene) seems to be a promising marker to measure *S. aureus* viability compared to rRNA (16S gene). Additional studies are needed for conformation and design of future clinical studies.

P011

Separate epidemics of *Chlamydia trachomatis* in men having sex with men compared to heterosexuals

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Introduction: Based on low resolution ompA typing, *Chlamydia trachomatis* (CT) genovar distributions differ between heterosexuals and men having sex with men (MSM). Heterosexuals mainly have infections with genovars D, E and F, whereas genovars D, G and J are found predominantly among MSM. No studies have been published so far that investigate clonal relationship using high resolution typing methods to characterise these differences. In this study, we aimed to investigate the cluster distributions of chlamydial strains among MSM and heterosexuals in Amsterdam.

Methods: Between July 2008 and May 2010, CT positive samples from heterosexuals and MSM were consecutively collected at the STI outpatient clinic in Amsterdam. Samples were typed for CT by multilocus sequence typing (MLST) from which minimum spanning trees were generated. Clusters were assigned allowing single locus variance (SLV) within one cluster. Clusters and their correlation with epidemiological data were analysed.

Results: In total, we were able to type 547 CT positive samples (78%); 256 samples from heterosexuals and 291 samples from MSM. We identified 9 large clusters (8 to 137 samples) in the minimum spanning tree, containing 88% of all samples. Five clusters consisted predominantly (86%-100%) of heterosexuals. These clusters contained sequence types with all major genovars (D, E, F, I, J and K). The 4 other clusters (genovars D, G, J and L2b) were almost exclusively consisting of samples from MSM, as only 4 samples from heterosexuals were found in these clusters. The diversity among samples from the MSM clusters was much lower compared to that from the heterosexual clusters. Notably the LGV samples showed to be fully clonal. Among the remaining 28 singletons and 15 small clusters (2 to 4 samples), only 1 sample from an MSM was seen, again demonstrating the higher diversity of the heterosexual strain types.

Conclusion: This study shows that CT MLST matches with genovar typing studies, but adds much more detail with respect to the clonality of the strains. We successfully identified distinct clusters of CT associated with heterosexuals and MSM, irrespective of the genovars. Among MSM, only a limited number of clusters were found, which were dominated by single sequence types. The heterosexuals showed more heterogeneous clusters, consisting of various sequence types, connected by SLV. We therefore conclude that the epidemics of *Chlamydia*

trachomatis among heterosexuals and MSM are largely separate epidemics. Whether these differences in CT strain distributions between MSM and heterosexuals are caused by mixing patterns of the host or pathogen related factors like tissue tropism remains to be elucidated.

P012

Rectal carriage of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in hospitalised patients: increased yield of screening with the use of selective broth enrichment

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Introduction: Adequate laboratory methods for the detection of extended-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-E) are crucial in the prevention of nosocomial transmission of ESBL-E and appropriate antimicrobial therapy for ESBL-E infections. The use of broth enrichment in the laboratory detection of ESBL-E is considered an unresolved issue. This study aimed to evaluate the added value of selective broth enrichment for the detection of rectal carriage of ESBL-E in hospitalised patients.

Methods: In October 2011 an ESBL-E prevalence survey was performed in a Dutch teaching hospital. Rectal swabs were taken from all patients hospitalised on the day of the survey. Swabs were directly plated on a selective ESBL screening agar plate (EbSA, Cepheid), and subsequently placed in a selective tryptic soy broth, containing cefotaxime (0.25 mg/L) and vancomycin (8 mg/L) (TSB-VC). After 18-24 hours incubation (35-37°C) the EbSA agar plate was read and the TSB-VC was subcultured on an EbSA agar plate that was read after 18-24 hours incubation (35-37°C). Species identification and susceptibility testing was performed for all isolates that grew on either one of the EbSA agar plates using VITEK 2 (bioMérieux). For suspected isolates (MIC ceftazidime and/or MIC cefotaxime> 1 mg/L) the presence of ESBL was phenotypically confirmed with the combination disk diffusion method for cefotaxime, ceftazidime, and cefepime, both alone and with clavulanic acid (Rosco). Test results were considered positive if the inhibition zone around the disk was increased ≥ 5 mm for the combination with clavulanic acid. Genotypic confirmation of the presence of ESBL genes was performed with the Check-MDR CT103 microarray (Check-Points).

Results: Rectal swab data were available for 549 of 638 (86%) patients. Direct EbSA culture detected ESBL-E in rectal swabs from 20 (3.6%) patients. TSB-VC subculture increased the number of ESBL-E positive cultures to 27 (4.9%) (McNemar Chisquare = 5.14, p = 0.0233). *Escherichia coli* was the predominant ESBL-positive species identified (24/29; 83%)

Conclusions: The use of selective broth enrichment resulted in a substantial and statistically significant increase in the yield of ESBL-E screening in hospitalised patients. Broth enrichment is, therefore, considered indispensable for the reliable detection of ESBL-E.

P013

Validation of three Aspergillus PCR assays on broncho-alveolar lavage samples

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Introduction: Current methods for the diagnosis of invasive pulmonary aspergillosis in broncho-alveolar lavage (BAL) include direct microscopy, culture, and antigen assays. Recently, two commercial PCR assays for the diagnosis of Aspergillus in BAL became available: the Alert Aspergillus (Nanogen Inc, CA) and the MycAssay Aspergillus (Myconostica Ltd, UK). We compared these two commercial assays with an in-house PCR assay versus culture.

Methods: 24 BAL samples from immunocompromised patients were collected and tested by conventional fungal culture and stored at -20°C. DNA isolation consisted of 60 seconds bead-beating in a Roche MagNA-Lyser followed by DNA extraction on the Roche MagNA-Pure LC using the Large Volume extraction kit. All PCR assays were performed on an ABI prism 7500 Taqman Real-Time thermocycler and interpreted by investigators that were unaware of the culture results. The commercial tests were performed according the instructions of the manufacturers. The in-house assay consisted of a previously published PCR for the genus Aspergillus (targeting the 28SrRNA gene) combined with a *A. fumigatus* (Afm) specific PCR (targeting the Calmodulin gene) in a duplex realtime PCR. The Alert and the MycAssay PCRs were performed according the manufacurers instructions. Myconostica recommends their own DNA extraction procedure. Eight samples were tested with the Myconostica MycXtra Kit and the MagNA Pure method. As the PRC results were comparable, only MagNA Pure LC purified DNA was used in this comparison.

Results: Fungal culture was considered as the gold standard. 10/24 BAL samples contained Aspergillus in culture: seven samples contained Afm and one sample contained Afm plus A. flavus, one sample was culture-positive with A. terreus and one with A niger. Of the 14 samples that did not grow

Aspergillus, three samples contained Penicillium. With the in-house PCR assay 8/10 Aspergillus samples were found positive for Aspergillus: 3/10 were (correctly) identified as Afm and 5/10 as Aspergillus genus (with possibility of containing Afm). The Alert PCR identified only 5/10 Aspergillus-positive samples as positive, two samples were uninterpretable and 3 were negative. The MycAssay PCR was used on only 8 of these 10 samples. 3/8 samples tested positive, all other samples were all not interpretable due to a failed internal control. One Afm-positive sample turned out negative (or uninterpretable) in all PCR-assays. This sample contained only a single Afm colony in culture, and an Aspergillus antigen assay performed on the BAL was negative. Of the three samples containing Penicillium, all tested negative for Aspergillus with the in-house PCR. With the Alert Aspergillus Q-PCR, one was wrongly identified as Aspergillus. The MycAssay yielded uninterpretable data for all three. The eleven samples that were culture-negative were only tested with the Aspergillus in-house duplex. 9 were negative, 2 yielded positive results for Aspergillus.

Discussion: While none of the assays was 100% in concordance with culture, the in-house assay was the most sensitive with 8/10 positives identified correctly. The two commercial assays performed poorly with many inconclusive results.

P014

Borrelia burgdorferi activates the inflammasome via the TLR2-MyD88 pathway and elicits IL-1β driven murine Lyme arthritis

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The recognition of *Borrelia burgdorferi* sensu lato by immune cells of the host defense system is predominantly mediated by Toll-Like Receptor (TLR)-2. After recognition by monocytes, macrophages, or NK-cells, potent pro-inflammatory mediators, such as IL-1β are produced. To obtain bioactive IL-1β, pro-IL-1β has to be cleaved to obtain bioactive IL-1β by intracellular caspase-1, which in turn is activated by a protein platform called the Nod-Like-Receptor-family member (NLRP)-3 inflammasome.

IL-1β has been linked to the pathogenesis of Lyme arthritis often seen after *B. burgdorferi* infection. However, the precise pathways through which *B. burgdorferi* recognition leads to inflammasome activation and processing of IL-1β in Lyme arthritis are still not elucidated.

In the present study, we investigated the contribution of several pattern recognition receptors and inflammasome components in murine Lyme arthritis elicited by live *B. burgdorferi*. *B. burgdorferi* was injected intra-articularly in knee joints of wild-type, TLR2-/-, Myd88-/-, NOD1-/-, NOD2-/-, RICK-/-, NLRP3-/-, ASC-/-, and caspase-1-/- mice. To confirm the crucial role of IL-1, IL-1R-/- mice were included. Peritoneal macrophages and bone marrow derived macrophages were used for *in vitro* cytokine production and inflammasome activation studies. In addition, the joint inflammation was analyzed in synovial specimens and whole knee joints. We show that *B. burgdorferi*-induced murine Lyme arthritis is highly TLR2-MyD88 dependent and that NOD1/NOD2/RICK-pathway is less involved. In addition, we demonstrated that ASC/caspase-1-driven IL-1β is crucial for the induction and maintenance of *B. burgdorferi*-induced murine Lyme arthritis.

P015

Medical-grade honey does not reduce skin colonization at central venous catheter insertion sites of critically ill patients: a randomized controlled trial

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Introduction: Catheter-related bloodstream infections (CRBSI) associated with short-term central venous catheters in intensive care unit (ICU) patients are a major clinical problem. Bacterial colonization of the skin at the insertion site is a major etiological factor for CRBSI. The aim of this study was to assess the efficacy of medical-grade honey in reducing bacterial skin colonization at central venous catheter (CVC) insertion sites.

Methods: Prospective, single-center, open-label randomized controlled trial at the ICU of a university hospital in the Netherlands. Application of medical-grade honey in addition to standard catheter site dressing and disinfection with 0.5% chlorhexidine in 70% alcohol. Skin colonization was assessed on a daily basis prior to catheter site disinfection. Primary endpoint was colonization of insertion sites with > 100 colony forming units at the last sampling before removal of the catheter or transfer of the patient from the ICU. Secondary endpoints were quantitative levels of colonization of the catheter sites and stratification of results for catheter location.

Results: Colonization of insertion sites was not affected by use of medical-grade honey, as 44/129 (34%) and 36/106 (34%) patients in the honey and standard care group, respectively had a positive skin culture (p = 0.98). Median levels of skin colonization at the last sampling were 1 [0 - 2.84] and 1 [0 - 2.70] log CFU/swab for the honey and

control group, respectively (p = 0.94). Also when stratified for catheter location, no significant differences were observed for these outcomes. Median ICU length of stay was also not affected, 9 [4 -18] days and 9 [5 -16] days in the honey and standard care group, respectively (p = 0.67) as was median duration of catheter use, 5 [3 - 7] and 5 [3 -7] (p = 0.32). Gender, days of catheter placement, catheter location and catheter type were predictive for a positive skin culture. Correction for these variables did not change the effect of honey on skin culture positivity.

Conclusion: Medical-grade honey does not affect colonization of the skin at CVC insertion sites sites in ICU patients.

P016

Growth condition dependant cell surface proteome analysis of *Enterococcus faecium*

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Introduction: Over the last thirty years Enterococcus faecium (Efm) has become the third most common nosocomial pathogen in hospitals worldwide. The aim of current study was to obtain insight in cell surface expression of Efm proteins when grown in physiological and clinically relevant conditions.

Methods: Cell surface protein expression of a clinical blood stream Efm isolate, E1162, grown until mid-log phase in brain heart infusion medium including 0.02% bilesalts (BHI-bile), urine and biofilm was compared with BHI grown cells. After washing the cell pellet, the cell surface was "shaved"using immobilized trypsin in 50 mM sodium-bicarbonate for 45 minutes at 37°C. The protein fragment containing supernatant was subsequently further digested using trypsin and peptides were identified using tandem mass spectrometry. The identified peptides were matched against the EfmE1162 whole genome sequence. Finally, LocateP was used to predict the subcellular location of the identified protein. All growth conditions were performed in 6 biological replicates.

Results: For the different growth conditions a total of 15, 11, 13, 13 and 11 proteins, in at least three of the six biological replicates, were identified in BHI-bile, urine, biofilm, TSB 1% glucose, and BHI respectively. Prediction of the subcellular location of identified proteins revealed that for BHI-bile 84%, urine 67%, biofilm 76% and BHI 67% belong to either lipid anchored, n-terminally anchored, secreted, multi-transmembrane or LPxTG anchored group of proteins. In general, the expression of proteins at the cell surface was very comparable with 10 proteins, including 2 ABC transporters, being expressed and detected in at least four of the studied growth conditions. Differentially expressed proteins included the Penicillin binding protein 5 (PBP-5), which was not identified in urine, a hypothetical lipoprotein that was only identified in urine, and a hypothetical lipid anchored surface protein that was only identified in BHI-bile.

Conclusion: This study revealed that proteolytic shaving of Efm cells identified cell surface proteins expressed during growth under different conditions. Differentially expressed proteins are of special interest as they might be involved in the interaction of Efm with the human host and may represent interesting candidate targets for vaccine development. Functional characterization of these proteins will provide more insight in the adaptive mechanisms of this emerging pathogen.

P017

Catheter related blood stream infections in home parenteral nutrition patients with long term taurolidine catheter lock therapy are caused by microorganisms with normal taurolidine minimal inhibitory concentrations

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Introduction: Prophylaxis with an anti-microbial catheter-lock solution containing taurolidine (TauroSept®), which is instilled in the catheter after administration of home parenteral nutrition (HPN), reduces the incidence of catheter related bloodstream infections (CRBSI) in patients on HPN. Nevertheless, some patients still develop CRBSI. Selection of resistant microorganisms is a common phenomenon in long-term antimicrobial prophylaxis. We hypothesized that long-term use of taurolidine selects for microorganisms with increased minimal inhibitory concentrations (MICs) to this agent.

Methods: Taurolidine MICs were measured in bloodstream microbial isolates from HPN patients with CRBSI by broth microdilution largely based on EUCAST methodology and read after 18h (bacteria) or 24h (*Candida spp.*). CRBSI diagnosis was based on clinical symptoms of infection, positive blood culture (drawn from a peripheral vein and/or catheter), and absence of other causes of bloodstream infections.

Results: From January 2009 until April 2011, 17 patients in a cohort of 158 HPN patients using a taurolidine catheter lock developed either a single (11 patients) or multiple (6 patients) luminal CRBSIs, resulting in 0.4 infections per 1000 catheter days. The censored maximum number of catheter days was 810. Fifty-five percent of CRBSI's were monomicrobial, 45% were polymicrobial. Fifty-three CRBSI-causing microbial isolates obtained from 29 different CRBSI episodes were investigated: CRBSI-causing microorganisms were gram-positive bacteria (60%), gram-negative rods (25%) or *Candida species*

(15%). CRBSI-causing gram-positive bacteria were most frequently coagulase negative *Staphylococcus species* (n = 16) or *S. aureus* (n = 7) with taurolidine MIC values of 512 µg/ml or less in 50% of the isolates (MIC_{50}). The taurolidine MIC_{50} for *K. pneumoniae* and *E. coli*, the two most common CRBSI-causing gram-negative bacteria, were 256 (n = 5) and 512 µg/ml (n = 3), respectively. In comparison with bacteria, CRBSI-causing *C. albicans* isolates had relatively high taurolidine MICs (2048-4096 µg/ml). MIC values for taurolidine in CRBSI-causing microorganisms were similar to values reported in the literature.

Conclusion: Prophylactic taurolidine lock therapy up to 810 days does not select for micro-organisms with increased taurolidine MICs.

P018

Capnophilic *Streptococcus pneumoniae* ST162 and ST344 strains have a polymorphism in the MurF cell wall ligase

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Introduction: Although approximately 8% of all pneumococcal strains are capnophilic, i.e require CO_2-enriched growth conditions, the genetic cause of this CO_2-dependent growth behavior is unknown. In this study we examined the genetic background of naturally occurring capnophilic pneumococcal isolates.

Methods: We performed MLST analysis of 71 capnophilic *S. pneumoniae* carriage and invasive isolates for molecular epidemiology. The genetic trait responsible for *S. pneumoniae* capnophilic behavior in two genetic clusters was revealed by transposon and directed mutagenesis.

Results: Capnophilic pneumococci could be grouped in at least seven different genetic clusters, four of which are closely related to global clones recognized by the international antibiotic resistant pneumococcal molecular epidemiology network (PMEN). Transposon mutagenesis linked the CO_2-dependent growth defect of ST162 strains of the Spain^{9V}-3 clonal complex to a genetic polymorphism in the *murF* cell wall ligase gene. This MurF V179A mutation also explained the capnophilic growth phenotype of the Norway^{NT}-42 related ST344 strains, but appeared to be lacking in the five other identified capnophilic genetic clusters. Finally, our experiments showed that acquisition of novel non-capnophilic murF variants by ST162 and ST344 strains is linked with acquisition of the penicillin binding protein 2B (*pbp2B*) gene by a hitchhiking effect.

Conclusions: We have revealed a novel functional polymorphism in the *S. pneumoniae* MurF protein that explains the CO_2-dependent growth restriction of a significant proportion of capnophilic pneumococcal strains. Variation in the MurF amino acid sequence of capnophilic strains by recombinational events that are imposed by pneumococcal exposure to CO_2-poor environmental conditions could have major consequences for genome plasticity and evolution.

P019

Evaluation of commonly used serological tests for the detection of *Coxiella burnetii* antibodies in well-defined acute and follow-up sera

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Introduction: During the Dutch Q fever outbreak, involved laboratories used their one technique to diagnose and to confirm acute Q fever cases. In 2010 criteria were developed to support clinical decision-making based on PCR and serological test outcomes. Based on inter-laboratory evaluation PCR method was standardized. However, different serological tests were used, including Enzyme-linked Immunosorbent Assay (ELISA), Indirect Fluorescent Antibody Test (IFAT), and Complement Fixation Test (CFT). The aim of this study was to compare the three different serological tests used in acute Q fever diagnostics. Diagnostic performances of different tests for acute disease as well as the kinetics in sequential serum samples were analyzed.

Methods: *Coxiella burnetii* IgG phase I, IgG phase II and IgM phase II antibodies were measured using a commercially available Enzyme-linked Immunosorbent Assay (ELISA) (Virion/Serion), an Indirect Fluorescent Antibody Test (IFAT) (Focus Diagnostics), and a Complement Fixation Test (CFT) (Virion/Serion) in a unique collection of acute, convalescent and follow-up sera from 126 patients with acute Q fever diagnosed by positive Coxiella burnetii PCR in blood. A total of 433 serum samples were included, with the following distribution of samples per time point: 66 at t = 0 (time of diagnosis), 121 at t = 3 months, 121 at t = 6 months, and 125 at t = 12 months after diagnosis. As controls, 34 paired blood samples from patients suffering from other respiratory infections were tested as well.

Results: IFAT demonstrated IgM phase II antibodies in significantly more sera compared to ELISA. Twelve months after the diagnosis of acute Q fever, 83% and 62% of the sera were still positive for IgM phase II with IFAT

and ELISA, respectively. Therefore, definitive serological evidence of acute Q fever cannot be based on a single serum sample only in epidemic areas and should include both IgM and IgG antibodies. All tests were comparable in confirming acute Q fever using IgG phase II antibody detection in paired samples (at 0 and 3 months) from 62 patients: 100% IFAT, 95.2% ELISA and 96.8% CFT. No significant cross-reactivity to other respiratory infections was observed. IFAT demonstrated significantly more IgG phase I and IgG phase II antibodies in follow-up sera. Significant differences between IgG phase I detection were observed at all follow-up time points. In all time points IFAT was significantly more positive than ELISA or CFT. After 12 months, IFAT was positive 76% (95/125), ELISA 28% (35/125), and CFT 14% (14/125) (p = 0.00).

Conclusions: This study demonstrated that the three serological tests are equally effective in diagnosing acute Q fever. However, in follow-up sera, more IgG antibodies were detected by IFAT than by ELISA or CFT, making IFAT probably more suitable for epidemiological surveys or for pre-vaccination screening programs, but it has practical disadvantages.

P020

Rapid genotyping of cytomegalovirus in dried blood spots by multiplex real-time PCR assays targeting the envelope glycoprotein genes gB and gH

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Introduction: Genotyping of cytomegalovirus (CMV) is useful to examine potential differences in the pathogenicity of strains and to demonstrate co-infection with multiple strains involved in CMV disease in adults and congenitally infected newborns. Studies on genotyping of CMV in dried blood spots (DBS) are rare and have been hampered by the small amount of dried blood available.

Methods: In this study, two multiplex real-time PCR assays for rapid gB and gH genotyping of CMV in DBS were developed.

Results: Validation of the assays with 39 CMV positive plasma samples of transplant recipients and 21 urines of congenitally infected newborns was successful in genotyping 100% of the samples, with gB1 and gB3 being the most prevalent genotypes. Multiple gB and gH genotypes were detected in respectively 36% and 33% of the plasma samples. One urine sample from a newborn with symptomatic congenital CMV was positive for gB1 and gB2. DBS of congenitally infected newborns (n = 41) were tested using 9 µl of dried blood, and genotypes were detected in 81% (gB) and 73% (gH) of the samples, with gB3 being the most prevalent genotype. No clear association was observed of specific genotypes with clinical outcome.

Conclusions: The CMV gB and gH PCR assays were found to be rapid, sensitive for detecting mixed infections, and suitable for direct usage on DBS. These assays are efficient tools for genotyping of CMV in DBS of congenitally infected newborns.

P021

Real-time PCR versus viral culture on urine as a gold standard in the diagnosis of congenital cytomegalovirus infection

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Introduction: Cytomegalovirus (CMV) infection is the most common cause of congenital infection. Whereas CMV PCR has replaced viral culture and antigen detection in immunocompromised patients because of higher sensitivity, viral culture of neonatal urine is still referred to as the gold standard in the diagnosis of congenital CMV infection. The objective of our study was to compare real-time CMV PCR with shell vial culture on urine in the diagnosis of congenital CMV, in a multicenter design.

Methods: A series of neonatal urines (n = 340), received for congenital CMV diagnostics and routinely assessed with shell vial CMV culture, was retrospectively tested by real-time CMV PCR.

Results: The proportion of newborns found to be congenitally infected by real-time CMV PCR was 8.2% (28/340, 95% CI 5.6-11.8%), and 7.4% (25/340, 95% CI 4.9-10.8%) by rapid culture. When considering rapid culture as reference, real-time PCR was highly sensitive (100%), whereas sensitivity of rapid culture was 89.3% when considering real-time PCR as reference.

Conclusions: Our results, supported by analytical and clinical data on CMV DNA detection in neonatal urine, suggest enhanced sensitivity of recent PCR techniques when compared to viral culture. There is considerable rationale to favor real-time CMV PCR as a gold standard in the diagnosis of congenital CMV infection. A large-scale study combining both laboratory and clinical data is required to determine the exact time frame for sampling of neonatal urine when using real-time PCR.

P022

Implementing neonatal screening for congenital cytomegalovirus: addressing the deafness of policy makers

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Review/ opinion: Congenital cytomegalovirus (CMV) infection is an important public health problem with approximately 7 in 1,000 newborns infected and consequently at risk for hearing impairment. Newborn hearing screening will fail to detect this hearing impairment in approximately half of the cases because late onset hearing loss is frequent. Hearing impairment has profound impact on cognitive and social development of children and their families, determining most of the disease burden of congenital CMV infection. The potential value of newborn screening for congenital CMV is increasingly discussed. To date, many experts acknowledge the benefit of antiviral treatment in the prevention of hearing deterioration in newborns with neurological symptoms, and the benefit of early identification of late-onset hearing impairment by means of extensive audiological follow up of infected infants. These opinions imply that the potential of newborn screening for CMV would lie in the identification of the large proportion of asymptomatic congenitally infected newborns at risk for developing late-onset hearing loss. Experience with postnatal antiviral treatment of symptomatic newborns is encouraging, but has not been studied in asymptomatic congenitally infected newborns. A large-scale study on the safety and effectiveness of combined screening and antiviral therapy for congenital CMV infection is the necessary next step to take and should not be delayed.

P023

A highly specific protease-based approach for the detection of *Porphyromonas gingivalis* in the diagnosis of periodontitis

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Porphyromonas gingivalis is associated with the development of periodontitis. Recently we developed a highly specific protease-based diagnostic method for the detection of *P. gingivalis* in gingival crevicular fluid. Screening of a proteolytic peptide substrate library, including fluorogenic dipeptides that contain D-amino acids, led to the discovery of five P. gingivalis-specific substrates. Due to the presence of lysine and arginine residues in these substrates it was hypothesized that the cleavage was mediated by the gingipains, a group of *P. gingivalis* specific proteases. This hypothesis was confirmed by the observation that *P. gingivalis* gingipain knock-out strains showed a clearly impaired substrate cleavage efficacy. Further, proteolytic activity on the substrates was increased by the addition of the gingipain stimulators dithiothreitol (DTT) and L-cysteine and decreased by the inhibitors leupeptin and N-ethylmaleimide.
Screening saliva and gingival crevicular fluid of periodontitis patients and healthy controls demonstrated the potential of the substrates to diagnose the presence of *P. gingivalis* proteases. By using paper points, a sensitivity of approximately 105 CFU/mL was achieved. *P. gingivalis* reactive substrates, fully composed of L-amino acids, and Bz-L-Arg-NHPhNO2 (L-BApNA) showed a relatively low specificity (44 - 85%). However, five *P. gingivalis* specific substrates, that each contained a single D-amino acid showed high specificity (96-100%). This observation underlines the importance of the presence of D-amino acids in substrates used for the detection of bacterial proteases of the gingipain family. We envisage that these substrates may improve the specificity of the current enzyme-based diagnosis of periodontitis associated with *P. gingivalis*.

P024

Flushing a toilet generates aerosols that can contain bacteria

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Introduction: Toilets produce aerosols during flushing. During the development and production of toilets, companies have to comply to international criteria regarding aerosols. We hypothesized that pathogenic bacteria might spread into the hospital environment through this route and subsequently colonize patients. In order to test our hypothesis we artificially contaminated three different toilets and analyzed the amount of aerosol droplets as well as the number of bacteria in these aerosols.
Methods: Three toilets, two ceramic (Villeroy and Boch, Germany) and one stainless steel (Franke, Belgium), were tested on aerosol formation by using a Perspex plate (500 x 500 mm) according to internationally accepted methods. After flushing, droplets with the size of 3-5 mm and> 5 mm, respectively, were counted on the lower surface of the Perspex plate. For each toilet this was done with increasing flushing water volumes (from 1 to 10 litre in steps of 1 litre). Bacterial numbers in aerosol droplets were analyzed by using Chromogenic U.T.I. Clarity Agar plates (Oxoid, Cambridge, UK) which for one minute were placed in

the centre of the toilet seat, surface down, and fixed with masking tape. Before flushing, toilets were contaminated with a 100 ml suspension containing 4 bacterial species (*Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis*) in a concentration of 108-9 colony forming units/ml. After overnight incubation of the agar plates (35°C) the number of grown colonies were counted.

Results: Aerosol droplets were observed on the lower surface of the Perspex plate after flushing each toilet. Droplet numbers raise with increasing amounts of flushing water volume. Droplet counts of both ceramic toilets were comparable. The stainless steel toilet produced five times more aerosol droplets compared to the ceramic toilets. Most droplets were sized 3-5 mm. With flushing water volumes less than 5 litre each toilet produced only 1 droplet of> 5 mm. However, when flushing water volumes increased the numbers of both 3-5 mm and> 5 mm droplets also increased. Results with both Perspex plate method and UTI-plate method correlated very well for each type of toilet.

Conclusion: Flushing a toilet generates aerosols that can contain pathogenic bacteria. The number of aerosol droplets depends on the type of toilet as well as the volume of flushing water that is used.

P025

A validation procedure for (new) toilets: how clean is clean?

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Introduction: Toilets may serve as a transmission source for pathogenic bacteria, which can lead to infection and colonization of persons. For hospitalized patients this may result in serious risks especially when they are immunocompromised. However, there are no data which define the minimal requirements of cleanliness for sanitary equipment on high care wards in hospitals.

We analyzed the conditions that are necessary for a significant reduction of bacteria from artificially contaminated toilets in order to create a validation procedure for (new) toilets.

Methods: According to Dutch legislation, disinfectants (biocides) need to reduce bacterial loads ≥ 5 log in order to be registered. We used this criterion to test three toilets, two ceramic (Villeroy and Boch, Germany) and one stainless steel (Franke, Belgium), with regard to their housekeeping characteristics after flushing and cleaning, respectively. We contaminated toilets with a 100 ml suspension containing 4 bacterial species (*Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis*) in a concentration of 108-9 colony forming units/ml. Culture swabs were used to sample each toilet before flushing, after flushing, and after cleaning, respectively. Each swab was placed in a tube containing 1 ml of saline. The tube was vortexed for 20 seconds. From each tube we made a series of 10-fold dilutions in saline. We inoculated 100 ml from each dilution tube on Chromogenic U.T.I. Clarity Agar plates (Oxoid, Cambridge, UK), and grown colonies were counted after incubation at 35°C for 18 hours.

Results: For each toilet we were able to demonstrate ≥ 2 log reduction of the initial contamination load after flushing. Additional cleaning adds ≥ 3 log reduction. Therefore the criterion of ≥ 5 log reduction was reached for each toilet. Supposed that toilets are properly cleaned, the three different toilets we tested all apply to the requirements of disinfection after flushing and cleaning.

Conclusion: The criterion of ≥ 5 log bacterial reduction of highly contaminated toilets seems to be a reliable parameter for disinfection and can be used for the validation of (new) toilets.

P026

Images of aerosols from toilets

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Introduction: During a research project on the contribution of aerosols that may cross-contaminate patients with pathogenic bacteria from toilets we intended to demonstrate aerosols from toilets by use of high-speed photography.

Methods: A Nikon D3 high-speed camera (diaphragm 11, shutter time 1/8000 of a second, lens 24-70mm) was placed in a fixed position in combination with three Nikon Speedlight SB-800 flashes. Aerosol droplets were enlightened by backlight whereas the background of the room was not enlightened.

We used three toilets, two ceramic (Villeroy and Boch, Germany) and one stainless steel (Franke, Belgium). Pictures were taken with various time intervals after the flush button was pressed.

Results: We were able to demonstrate the presence of aerosols from each of the three toilets. Aerosols could only be demonstrated 1-2 seconds after flushing. Thereafter no aerosol droplets were visible anymore. There were remarkable differences in droplet densities between the toilets, in favour of the ceramic toilets, which corresponded very well with previous test results (see other abstract). Differences in toilet design, the finishing process, as well as the choice of materials probably contribute to the

different results. It is a challenge to create toilets that are appropriate for medical care which are safe with regard to infectious hazards.
Conclusion: Aerosols are present after flushing the toilet and these aerosols can be visualized by use of high-speed photography.

P027

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) with the BD-MAX™ system

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Introduction: The BD-MAX (Becton Dickinson) is a fully automated molecular platform for the extraction of nucleic acids from different kinds of specimens followed by real-time PCR. The system can be used for BD developed IVD/CE assays. In addition, the BD-MAX is an open system which allows users to run their laboratory designed assays (LDA).
In this evaluation, detection of MRSA from broth-enrichment culture using the BD developed MRSA assay for the BD-MAX is compared to sub-culture on chromogenic agar.
Methods: ESwab (COPAN) samples (mainly throat, nasal and perineum) with the request for MRSA culture were prospectively collected.
The swabs were transferred into a Trypticase Soy Broth and subsequently incubated for 18-24 hours at 35°C. After incubation,10 µl of broth was transferred to the sample buffer tube (BD) and tested directly in the IVD/CE MRSA-assay on the BD-MAX. Besides this direct testing, also10 µl of the incubated broth was used to inoculate a ChromID MRSA agar (bioMerieux) and a blood-agar plate. Suspected colonies were followed by Staphaurex (Murex), MALDI-TOF MS (Brukner) and VITEK2 (bioMérieux) analysis for MRSA identification and antigen resistant testing.
Results: In total 325 samples were tested on the BD-MAX system. Thirty four samples (10.5%) were tested positive. Interestingly, these positives could be divided into two groups: 1) low Ct value group (n = 22) with Ct values between 13 and 29 (mean Ct = 20); 2) high Ct value group (n = 12) with Ct values between 30 and 38 (mean Ct = 35). Surprisingly, only the low Ct value positive samples were culture positive on the ChromID MRSA agar. The group with high Ct values were retested in the BD-MAX and also cultured again from broth and from sample buffer tube. The results in the BD-MAX were reproducible and again none of the high Ct-value positive samples were culture-positive, indicating a higher sensitivity of the PCR. The clinical relevance of these additional high Ct-value positives has to be investigated. No samples were culture positive and PCR negative. If culturing is taken as the "gold standard"and based on the 325 samples that were tested on the BD-MAX system the total agreement is 96%. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are 100%, 96%, 65% and 100% respectively.
Conclusion: The IVD/CE MRSA assay on the BD-MAX is a highly sensitive method to detect MRSA bacteria from broth-enrichment ESwab cultures. However, the clinical value of the positive samples with high Ct needs to be elucidated. The BD-MAX system is a truly walk-away system is user friendly and fits well in a microbiological laboratory setting.

P028

Validation and update of a prediction rule for the automated surveillance of drain-related meningitis complicating external cerebrospinal fluid drainage

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Introduction: Healthcare-associated infections (HAI) result in increased patient morbidity and mortality. Traditional manual surveillance for HAI is time-consuming and error-prone. Therefore, an automated surveillance system was previously developed using drain-related meningitis (DRM) as a prototype infection [1]. In this study, the model was validated on an independent patient population and model update was performed.
Methods: Model validation was performed on patients receiving an external ventricular or lumbar drain in the UMC Utrecht between January 1st 2010 and June 10th 2011 (n = 137). Children, patients with multiple simultaneous drains, < 1 day of follow-up or meningitis at drain placement were excluded (n = 105 in analysis). The model uses drain characteristics, results from clinical chemistry and microbial culture and antibiotic use. Model prediction was compared to routine surveillance of DRM by two infection control professionals (reference standard). Missing data were imputed using multiple imputation. Subsequently, model update was performed on all patients (development and validation set, 2004 - 2011, n = 881) to include newly available predictors (Gram stain, urgency of admission and destination after discharge) and optimize model performance for future patients. Similar exclusion criteria were applied (n = 653 in analysis). All previous variables along with the new variables were considered in logistic regression. Shrinkage through bootstrapping was performed to maximize the generalizability of the model. The resulting model was analyzed for discriminatory performance and group-level predictions (calibration-in-the-large).

Results: In the validation sample, 20 of the 105 patients developed DRM as determined by the reference standard (17.4/1000 days at risk). The area under the ROC curve, a measure of discrimination, was 0.951 (95% confidence interval (CI): 0.914 to 0.988). The sensitivity and specificity were 100% and 88.2% respectively, and predictive values were 58.8% positive and 100% negative. The predicted overall number of infections was 19.52 (observed = 20). The updated model now includes Gram-stain results. The area under the ROC curve of the updated model is 0.966 (95% CI 0.950 to 0.982). If a predicted probability cut-off of 0.06 is chosen, the number of charts that requires manual review for confirmation is reduced from 653 to 198 (30%) with 99.0% sensitivity. However, if a small loss in sensitivity is accepted (for example 95.1%), efficiency increases and 31 less charts require manual review (75% vs. 70% workload reduction). Group level prediction, in which predicted probabilities are summed without application of a cut-off, was adequate over the years.

Conclusion: From the results presented above it can be concluded that:

1. The use of multivariate prediction models is feasible for the automated surveillance of healthcare-associated infections, with drain-related meningitis as a proof-of-principle
2. Since this retrospective surveillance does not have implications for the treatment of individual patients, a small decrease in sensitivity can be deemed acceptable in return for increased efficiency of surveillance.

References:

1. van Mourik MS, Groenwold RH, Berkelbach van der Sprenkel JW, et al. Automated detection of external ventricular and lumbar drain-related meningitis using laboratory and microbiology results and medication data. PLoS One. 2011;6:e22846.

P029

A prolonged hospital outbreak with Metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa (PA)* in a Burn Centre and Intensive Care Unit linked to an environmental reservoir

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Background: A prolonged outbreak with a VIM-2-producing PA strain among 22 patients admitted to the intensive care (ICU) unit or Burn Centre (BC) of a regional teaching hospital in the Netherlands was investigated.

Methods: A case was defined as laboratory-confirmed infection with a strain of PA resistant to meropenem, imipenem and ciprofloxacin (MBL-PA). We conducted an epidemiological investigation, case-control studies, molecular genotyping, performed surveillance cultures from hospitalized patients and health care workers (HCWs) and environmental cultures.

Results: Between February 2006 and March 2011, 22 cases of MBL-PA were identified, 14 patients admitted to the BC and 8 patients admitted to the ICU. The affected patients were aged 36 to 93 years (mean 62 years), 12 males and 10 females. Molecular typing showed that strains were genetically identical and carried blaVIM2. All surveillance cultures from HCWs tested negative. Environmental cultures were positive for MBL-PA in the sink plughole in ICU room 1 and BC room 4. A stay in ICU room 1 or BC room 4 was found to have a strong association with the acquisition of a MBL-PA strain, with an odds ratio of 75 (95% CI, 4 to 1434) and 6 (95% confidence interval, 1 to 38), respectively. The affected sinks were decontaminated and the siphons replaced, and a decontamination policy for all sinks on both wards was implemented. To date, no new cases of MBL-PA colonisation or infection have been identified.

Conclusions: In this study, acquisition of the outbreak strain was significantly associated with exposure to two patient rooms. Although the environment serves as a reservoir for a variety of micro organisms, it is rarely implicated in disease transmission. We stress the importance of the environment as a potential reservoir for infections with multidrug resistant pathogens such as MBL-PA.

P030

Evaluation of three enzyme immunoassays and a loop-mediated isothermal amplification assay for the laboratory diagnosis of *Clostridum difficile* infection

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Introduction: The laboratory diagnosis of *Clostridium difficile* (*C. difficile*) infection (CDI) consists of the detection of toxigenic *C. difficile*, and/or its toxins A or B in stool, for which a variety of methods is available. We assessed the performance of three toxin enzyme immunoassays (EIAs), a *C. difficile* specific glutamate dehydrogenase (GDH) antigen EIA and a commercial toxin gene amplification assay in relation to toxigenic culture.

Methods: In a prospective study of five months, stool samples from patients with suspected *C. difficile* infection and/or CDI-associated risk factors, were cultured for

toxin producing *C. difficile* and tested with three toxin EIAs: ImmunoCard Toxins A & B (Meridian Bioscience, Cincinnati, OH), Premier Toxins A & B (Meridian) and C. diff Quik Chek Complete (TechLab, Blacksburg, VA) which contains both a toxins A and B test and a GDH test, and the loop-mediated isothermal amplification assay illumigene *C. difficile* (Meridian). Culture negative samples that were positive in the illumigene assay were retested using two real-time PCRs targeting tcdA and tcdB respectively. The gold standard was defined as isolation of a toxin producing *C. difficile* from stool samples.

Results: In total 986 samples were analyzed, of which 73 (7.4%) were toxigenic culture positive. Four samples gave a repeat invalid result in the illumigene assay. These were negative in all other assays. Of three samples that were culture negative and illumigene positive, two were positive for tcdA and tcdB when tested with real-time PCR. Sensitivities of the toxin tests Immunocard, Premier and C. diff Quik Chek were 41.1%, 41.1% and 54.8% respectively, specificities were 99.0%, 98.9% and 100% respectively. The illumigene assay showed sensitivity of 93.2% and specificity of 99.7%. The rapid GDH test showed sensitivity of 97.3% and a negative predictive value (NPV) of 99.8%.

Conclusion: Of the *C. difficile* toxin tests, the best performance was obtained for the C. diff Quik Chek Complete assay. The high NPV of the GDH assay supports the implementation of a two-step algorithm with a GDH assay as screening test. In a laboratory without PCR facilities, the illumigene assay will be a good alternative molecular method.

P031

Is otitis externa with *Pseudomonas aeruginosa* caused by a contaminated ear syringe?

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Introduction: Otitis externa is often caused by *Pseudomonas aeruginosa* (*P. aeruginosa*). When infection is suspected, a general practitioner (GP) will examine the outer ear with an otoscope and will perform an ear lavage using a syringe if necessary. Pus from the ear canal is sent to the microbiology laboratory for investigation. A GP in our region noticed that patients sometimes returned shortly after their ear lavage, with an otitis externa caused by *P. aeruginosa* and wondered about a causal relationship, *P. aeruginosa* was subsequently also cultured from an ear syringe in his practice. In a study among general practices we assessed whether cross-contamination with *P. aeruginosa* between patients could have occurred by using contaminated otoscopes or ear syringes.

Methods: In 2010, all strains of *P. aeruginosa* isolated from ears of patients were kept stored. Visiting general practices with *P. aeruginosa*-positive patients, we swabbed otoscopes and ear syringes and interviewed GPs and nurses about the use and cleaning of the instruments. The *P. aeruginosa* isolates cultured from instruments and those from patients were genotyped. We asked the GPs to check the records of positive patients for an ear lavage prior to the otitis externa, and of 100 other patients who underwent lavage earlier whether they had developed an otitis externa subsequently.

Results: In eight of the 17 practices *P. aeruginosa* was cultured from ear syringes. In three practices a patient strain and a syringe isolate were found to be identical. Two patients had had a lavage a few weeks before their otitis. In two other practices several patients were found to share identical strains. The interviews showed that disposable otoscope parts were reused and that syringes were rarely disinfected. Of the 55 otitis patients, 11 had had a lavage less than two months before. Of these, two patients had an identical strain as was found on an ear syringe. Of 100 patients who had had a lavage in 2011, three returned to the practice with an otitis externa, but no *P. aeruginosa* was isolated.

Conclusion: Genotyping results of *P. aeruginosa* from ears and, respectively, instruments showed that transmission between an ear syringe and a patient's ear is possible during ear lavage. Ear syringes are often insufficiently cleaned. Based on these results we made recommendations how to use and clean otoscopes and ear syringes in the general practice, in order to prevent transmission of pathogenic microorganisms.

P032

Evaluation of automation and Lean methodologies on the urine workflow in a microbiology laboratory

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Introduction: Urinalysis remains one of the three major *in vitro* diagnostic screening tests after serum chemistry profiles and complete blood counts. When it comes to improving efficiency, the greatest benefits often come from revamping high-volume manual processes. Traditional, manual urine screening methods are time-consuming, outmoded and inefficient and open to significant errors that negatively impact patient care and drive up costs. Inaccurate microscopy results may also lead to unnecessary cultures and a subsequent cost. Estimates have shown that 25 percent of urine cultures are unnecessary, which not only waste resources, but delays other results

A "top to bottom"assessment of urinalysis testing, that is, pre-analytical, analytical, and post-analytical work

processes and outcomes were evaluated and addressed, using Lean Six Sigma methods and automation.

Methods: A baseline measure of turnaround time (TAT) defined as time of booking in to time the report is released and staff productivity was established. This was compared to TAT performance and staff productivity after implementing Lean process modifications, included the creation of a urine work cell, automation for the analytical and post-analytical stages. Five LEAN principles were applied to the urine work-flow:

Results:

- Average time required for a technician to process a urinalysis specimen was reduced by 50 percent, from 150 seconds to 75 seconds. This was predominately due to elimination of non-value steps in the process.
- Prior to Lean Six Sigma and automation average turnaround times were 36.77 hrs and post it improved dramatically to 0.72 hrs. This equates to a 5000% improvement.
- Staffing mix on the urine bench changed substantially. This enabled the redeployment of more skilled staff, improving overall lab performance.

Conclusion: Implementation of Lean Six Sigma methodologies in the urine work flow of a microbiology lab resulted in significant improvements in both productivity and TAT. This was accompanied by more accurate results, reduction in the number of enquires regarding specimen status and an enhanced ability in the lab to meet peaks in demand. Implementation of automation and Lean demonstrated synergy between the two. Lean improved the processed while automation standardized the process. Lean eliminated the waste while automation automated the processes that helped reduce the waste. Ultimately the two significantly helped improve patient care pathways.

P033

Validation of new liquid faecal swab for the detection of *Clostridium difficile* from faecal specimens with multiple diagnostic assays

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Introduction: Pathology labs are being faced with the challenge of doing more with less money. This has led to a dramatic surge in innovative ideas and products. The pre-analytical process has been targeted particularly as significant improvements in efficiencies can be made here. The majority of front-end solutions require liquid base approach to the collection of samples which has led to the marketing of various forms of liquid swabs. The latest foray into liquid based microbiology comes from MW&E and its new liquid medium faecal swab. The goal was an innovative specimen collection device that would automatically transform the sample into liquid in standardized containers containing medium specific for enteric pathogens. This study was carried out to determine the compatibility of the liquid faecal swab with assays which detect *C. difficile* toxins A/B via an ELISA approach, GDH (Glutamate Dehyrogenase) and a molecular test.

Methods:

- 50 faecal specimens, of which 49 had tested positive previously for *C. difficile* direct from a faecal pot were used to validate the liquid faecal swab device.
- The foam tipped swab of the collection kit was used to sample the clinical material.
- The commercial kits used in the validation were: C.DIFF QUIK CHEK® by TechLab (A rapid test for the detection of C.difficile Glutamate Dehyrogenase); VIDAS® C.difficile Toxin A & B by Biomerieux and the BD GeneOhm™ real time PCR assay by Becton Dickinson.
- Testing of the commercial kits where according to manufacturer's protocols.
- A negative faecal liquid swab was tested for each kit as a control and to determine if there was any interference from the liquid medium (modified Cary Blair).

Results:

- Of the 50 samples tested 49 were positive utilizing all the above named kits whether the sample was taken from the liquid faecal swab or direct from a traditional stool container.
- The foam swab collected on average 136mg of faecal material.

Conclusion:

- The dilution factor introduced by the liquid medium had no effect on the result. There was a 100% correlation from doing the assay direct from the stool or performing it from the faecal liquid medium.
- The Cary Blair medium had no effect on the PCR method. There was a 100% correlation between doing the PCR direct on the stool sample or straight from the inoculated Cary Blair.

P034

Transcriptomics of *Agaricus bisporus* reveals changes in carbon metabolism in different growth stages

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Carbon source utilisation is an important aspect of fungal physiology. Many fungi are exposed to mixtures of carbon sources, which enable them to make choices to use the most favourable substrate. Agaricus bisporus is commonly

grown on compost, which consists mainly of straw and horse manure. This means that the majority of the carbon source is present as plant-based polysaccharides, which themselves consist of many different monomeric components. The major components of these polysaccharides are glucose, xylose, and arabinose, while smaller amounts of galactose, galacturonic acid, rhamnose and mannose are also present.
In this study we evaluated the expression of genes involved in the catabolism of different sugars during different stages of growth of *A. bisporus*. Clear differences in the expression of genes from different catabolic pathways were observed between mycelium grown on plates, in compost or in casing-soil, and in fruiting bodies, suggesting a high level of specialization.

P035

Clinical Performance of three commercial tests for detecting *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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Objectives: The objective of this study was to compare the performance of both (1) the fully automated Roche cobas 4800 system and (2) the Diagenode real-time PCR kits for CT, NG and CT/NG to (3) the Roche COBAS TaqMan CT and COBAS TaqMan 48 - Diagenode NG tests for detecting Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG).
Methods: The study was performed using 245 clinical samples from routine diagnostics and consisted of 185 prospectively collected samples (60 urine and 125 swab specimens), and 30 CT and 30 NG positive, retrospective samples (10 urine and 20 swab specimens and 7 urine and 23 swab specimens, respectively). Results of the cobas 4800 and the Diagenode real-time PCR tests were compared to the COBAS TaqMan test results.
Results: For CT, sensitivities ranged from 83.3% for swabs in the cobas 4800 assay to 100% for urine samples in the Diagenode test. Specificities ranged from 99.1% - 100% for all CT tests. Negative predictive values ranged from 94.9% for swabs in the cobas 4800 assay to 100% for urine samples in the Diagenode test. Positive predictive values ranged from 96.9% to 100% for all CT tests. Discrepancies were in all cases samples that were weakly positive in the COBAS TaqMan assay (mostly swabs), and that were diluted prior to additional testing. For NG, all test results were 100% concordant.
Conclusions: Both the cobas 4800 system and the Diagenode real-time PCR tests showed good performance for screening of samples for C. trachomatis and N. gonorrhoeae compared to the COBAS TaqMan assays.

P036

Surveillance of non-O157 STEC isolates in the Netherlands 2007-2011

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Introduction: In the Netherlands, an intensified surveillance of STEC O157 has existed since 1999. In 2007, the scope of this surveillance was widened to include non-O157 STEC isolates.
Methods: For this purpose, a real time PCR method was developed in 2006 to test for the presence of shigatoxin genes directly in faecal samples. From 2007, laboratories were encouraged to use this method and submit up to five cultured isolates (sorbitol positive or negative) from a PCR positive faecal sample to the RIVM in an effort to obtain the isolate responsible for the positive signal. These isolates were tested for the presence of shigatoxin and other virulence genes and when found positive, they were further typed with serotyping. Over the years, more laboratories included a PCR in their routine diagnostic workup.
Results: During four years (2007-2010) of STEC surveillance, this strategy resulted in submission of 3536 isolates from 703 different patients. Only for 193 patients (27.5%), an isolate could be found harbouring either the shigatoxin 1 or 2 gene. When isolates harbouring virulence genes eae or hly are also included, this success rate climbs to 32.6% (229 patients). The preliminary results from 2011 indicate a significantly higher success rate (47.5%) for finding a shigatoxin gene positive isolate. In only 12 of these patients (6.2%), the isolate found was O157, but O157 is also submitted based on sorbitol negativity on SMAC, so this does not reflect the prevalence of O157 in the general population. Most frequently isolated serotypes are O63 (11.4%), O26 (10.9%) and O91 (7.3%). Remarkably, shigatoxin subtype 2f, which was added as a target for PCR in 2008, was found in 21.2% of all positive isolates. This subtype was first found in pigeons and is not (yet) widely regarded as a major pathogen. From four patients, an STEC isolate was obtained more than once, with two weeks to six months time between sampling dates.
Discussion: The increased success rate of isolating an STEC isolate in 2011 is most likely caused by a change in submission criteria. Because the success rate of isolation decreased as the Ct value found in the faecal samples increased, laboratories were requested not to submit random cultured isolates from samples with a high Ct value, i.e.> 35 in the beginning of 2011. Even though the number of found O157 isolates using this strategy does not

represent prevalence in the general population, it is clear that diagnostic methods should also include non-O157 STEC. However, the clinical relevance is not well-established for all non-O157 STEC serogroups. A new insight that this surveillance strategy has provided is the high occurrence of serotype O63, which carry subtype stx2f, and other STEC harbouring stx2f in the Netherlands. This shigatoxin subtype has also been described to be emerging in Germany but only occasionally in other countries.

P037

Comparison of the MALDI Biotyper system using two specimen processing protocols for the identification of bacteria from positive blood culture bottles

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Objective: For patients with a blood stream infection it is important to receive a targeted antibiotic therapy as soon as possible. In routine microbiology this is usually not available sooner than after a day of a blood culture becoming positive. In this study, we evaluate the commercially available Sepsityper kit (Bruker) and an in house protocol coupled with the MALDI Biotyper system (Bruker) for isolation and identification of micro-organisms directly from positive blood cultures.
Methods: Positive blood cultures (n = 69) from the Regional Laboratory of Public Health were prospectively collected between October and November 2011. Only initial positive cultures from each patient were enrolled to avoid duplicate analysis of the same septic episode. For isolating bacteria two methods were used: each specimen was processed using the commercial Sepsityper Kit method (1) and after gel separation of 5 ml of the positive blood cultures centrifuged in Vacutainer tubes (Vacutainer method, 2). The obtained bacterial pellet was applied to the Maldi target before and after the standard ethanol/formic acid extraction protocol. Identification was performed using the Maldi-tof MS, generated spectra were processed by the Biotyper 2.0 software which gave genus and species names along with a score expressing the likelihood of correct identification. All results were compared to the results of conventional determination protocols.
Results: Correct identification by both methods was significantly improved with the additional ethanol/formic acid extraction step which produced an increase from 16/69 to 40/69 and 37/69 to 49/69 for the Vacutainer method and Sepsityper Kit respectively. The Vacutainer method gave correct species names in 58% (40/69) of the cases whereas the Sepsityper Kit did in 71% (49/69). This difference is caused by the difference between the results of gram-negative and gram-positive samples. The results for gram-negative bacteria was 96,8% (30/31) correct identification for the Vacutainer method and 93,5% (29/31) for the Maldi Sepsityper kit. Adversely, of the Gram positive samples the Vacutainer method correctly identified 26,3% (10/38) whereas the Sepsityper Kit identified 52% (20/38) of samples correctly.
Conclusion: Bacterial identification using the Maldi-Tof MS is a fast and potential useful technique. In general, the Sepsityper Kit gives a higher percentage of correct identification on species level compared to the Vacutainer method. However, for Gram negative micro-organisms the Vacutainer method is an easy to use, cheap alternative for the more labor intensive Sepsityper Kit method.

P038

Characterization of YkuE, a substrate of the *Bacillus subtilis* Twin-arginine translocation system

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Introduction: The twin-arginine translocation pathway is dedicated to the transport of fully folded proteins across the cytoplasmic membranes of many bacteria and the chloroplast thylakoidal membrane. This system has the unique ability to specifically recognize proteins with a twin-arginine motif in their signal peptide and, more importantly, to discriminate between folded substrate proteins and unfolded proteins that are not accepted. The identification of its substrates, however, still remains a difficult task. Some of the bacterial substrates that were identified in early studies possess a metal cofactor that needs to be inserted into the folded protein prior to translocation but also cofactor-less proteins can also be transported in a Tat-dependent way. In addition, these proteins are involved in various different cellular processes and hence predictions based just on these characteristics are not reliable. Nevertheless, a combination of this type of functional information and computer algorithms that recognized the main characteristics of the twin-arginine signal peptide can provide very important hints for the identification of new Tat substrates. In the present studies, we focused our attention on the Tat system of *Bacillus subtilis*, a well-known model organism that has a high capacity to secrete proteins into the external milieu. Recently it was shown that the *B. subtilis* YkuE protein, a putative metallophosphoesterase, possesses a twin-arginine motif that is able to promote the Tat-dependent secretion of an agarase in Streptomyces coelicolor (Widdick, D.A. et al., 2008, J. Mol. Biol. 375:95-603). We decided to study the YkuE protein in its original host *B. subtilis* in order to confirm its putative Tat-dependence

and get more information on the predicted metal cofactor bound to it.

Methods: To this purpose, wild-type and tat mutant cells of *B. subtilis* were fractionated and analyzed trough SDS-PAGE and Western blotting. Moreover, a Strep-tagged variant of YkuE was overproduced in *B. subtilis*, purified and subjected to inductively-coupled plasma mass spectrometry (ICP-MS) analyses in order to determine quantitatively the content of bound metal species. The putative catalytic property of the protein was also tested with an enzymatic assay for phosphoesterase activity.

Results: We now show that YkuE is a substrate of the *B. subtilis* Tat system that localizes to the cell wall of this bacterium. We also show that the protein has a binuclear Mn/Zn metal center with metallophosphoesterase activity.

Conclusion: The Tat-system of *B. subtilis* is able to transport proteins to different cellular compartments including the cell wall. YkuE has a phosphoesterase activity that depends on a binuclear Mn/Zn metal center. Further detailed investigations of the catalytic properties may include the closer inspection of its phosphoryl substrate specificity as well as the detailed analysis of the metal binding site by metal replacement analyses and binding site mutagenesis.

P039

Variability of ESBL genes carried by hospitalised patients

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Introduction: Worldwide Extended Spectrum Beta-Lactamase producing *Enterobacteriaceae* (ESBL-E) are spreading rapidly, in both the hospital and the community. Different ESBL genes have been associated with reservoirs in humans ($bla_{CTX\text{-}M\text{-}15}$) and animals ($bla_{CTX\text{-}M\text{-}1}$ and $_{blaTEM\text{-}52}$). Studying the variability of ESBL genes in humans is important to monitor changes and/or emergence of potential reservoirs of ESBL. This study presents the variability of ESBL-genes carried by hospitalised patients in the Netherlands.

Methods: Two prevalence surveys were performed in autumn of 2010 and 2011 in a Dutch teaching hospital. Rectal swabs were taken from all patients hospitalised on the day of the survey, including day-care patients. Swabs were directly placed in a selective tryptic soy broth enrichment, containing cefotaxime (0.25 mg/L) and vancomycin (8 mg/L) (TSB-VC) and incubated overnight. After 18-24 hours incubation (35-37°C) the TSB-VC was subcultured on a selective ESBL screening agarplate (EbSA,Cepheid), that was read after another 18-24 hours of incubation (35-37°C). Species identification and susceptibility testing was performed for all oxidase negative gram-negative isolates that grew on the EbSA agar using Vitek 2 (bioMérieux). For isolates with an MIC for ceftazidime and/or cefotaxime > 1 mg/mL the presence of ESBL was confirmed using the combination disk diffusion method for cefotaxime, ceftazidime and cefepime, both with and without clavulanic acid (Rosco). Characterisation of the ESBL genes was performed for all phenotypic ESBL positive isolates using the Check-MDR CT103microarray (Check-Points).

Results: Rectal swabs were obtained from 559 of 668 (84%) and 572 of 638 (90%) hospitalised patients in 2010 and 2011, respectively. Genotypic testing confirmed the presence of ESBL-E in 23 patients with 23 ESBL-E and 27 patients with 30 ESBL-E in 2010 and 2011, respectively. In 53 ESBL-E the following genes were found: 38 isolates with CTX-M1 group (n = 21 $_{blaCTX\text{-}M\text{-}1}$, n = 14 $_{blaCTX\text{-}M\text{-}15}$, n = 1 $_{blaCTX\text{-}M\text{-}3}$ and n = 3 $_{blaCTX\text{-}M\text{-}32}$), 8 isolates with CTX-M9 group, 5 isolates with SHV (238S+240K) and one isolate with TEM (104K+238S). CTX-M genes were the most frequently observed ESBL genes (87%).

When comparing the distribution of ESBL genes between the two surveys a remarkable increase is observed in the prevalence of $_{blaCTX\text{-}M\text{-}15}$: 17% (4/23) in 2010 vs. 33% (10/30) in 2011. Compared to a similar survey performed in 2009 the prevalence of $_{blaCTX\text{-}M\text{-}15}$ increased statistically significant (from 13% (16/46) in 2009 to 33% (10/30) in 2011; p = 0.042). (2009 data published by Overdevest et al. in Emerg Infect Dis 2011;17:1216-22).

Conclusion: This study shows a predominance of CTX-M genes (87%) in ESBL-E cultured from hospitalised patients, in particular $_{blaCTX\text{-}M\text{-}1}$ and $_{blaCTX\text{-}M\text{-}15}$. A substantial and statistically significant increase in the prevalence of blaCTX-M-15 was found from 2009 to 2011. The epidemiological implications of this finding are currently unclear. However, it may be indicative of the increasing importance of the human reservoir.

P040

An investigation of the suitability of liquid transport medium for recovery of enteric pathogens from faecal specimens

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Introduction: In recent years a number of automated processing systems have been introduced into clinical microbiology laboratories. These systems require a liquid specimen such as blood or urine as the matrix for processing. Transport swabs are also available with liquid medium for respiratory, urogenital, and wound specimens. Faecal specimens, however, which account for a considerable proportion of specimens in a routine clinical

laboratory, could not be processed unless first emulsified and suspended in a broth. Recently a transport swab for faecal specimens (Faecal Transwab®) has been developed which at the time of collection converts faecal specimens into a liquid specimens; suitable for direct processing on automated platforms. The present study was devised to investigate the performance over typical transport periods of this new device with a range of important enteric pathogens.

Methods: The Clinical and Laboratory Standards Institute standard M40-A describes methods for assessing the ability of transport devices to maintain various microorganisms in a viable condition for up to 48 hours during transport at ambient or refrigerated temperatures. The standard, however, does not include any enteric pathogens. The present study used the principles and methods of CLSI M40-A to evaluate the new device, adapted for the enteric microorganisms which are the target for faecal swabs.

Stock Test suspensions were prepared for *Escherichia coli* 0157, *Campylobacter* spp, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*, *Vibrio cholerae*, *Salmonella Typhi*, *Salmonella Typhimurium*, and *Salmonella Enteritidis*. Swabs in triplicate were inoculated with dilutions of each microorganism, and held for 0, 24 and 48 hours at ambient temperature and refrigerated temperature. After the holding period aliquots of the transport medium were inoculated on to plates of the appropriate agar medium, incubated, and any colonies were counted.

Results: Acceptable recoveries within the parameters for CLSI M40-A were recorded for all organisms at refrigerated temperature, at the ambient temperature over the 48 hour period a decline in the number of colonies was observed.

Conclusion: This investigation has shown that Fecal Transwab® device can efficiently recover a range of enteric pathogens at refrigerated temperature during simulated transport conditions over a 48 hour period, in compliance with the principles of CLSI standard M40-A.

P041

Detection of *Borrelia*-specific immune complexes in patients with Erythema migrans

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Introduction: The *Borrelia burgdorferi*-specific immune complex (IC), if detectable, can be a possible early marker of active infection. ICs can be precipitated with polyethylene glycol (PEG) in order to isolate them from the serum of Lyme patients.

Methods: Immune complexes were isolated from the serum samples of n = 40 Erythema migrans (EM) patients and were tested at onset before and at 12 weeks after the start of antibiotic treatment. The original serum sample and the dissolved ICs were then analysed by a recomLine *Borrelia* IgM and IgG immunoblot.

Results: In approximately 40% of the EM patients ICs were detected in the serum samples both at onset and at 12 weeks thereafter. IgM ICs were mainly detected against p41 (flagel) and the OspC antigen and IgG ICs were only rarely detected. Some patients however showed a clear seroconversion for *Borrelia* specific ICs on follow up. Interestingly in one EM patient with reported persistent symptoms after treatment ICs were also detected at 12 weeks against p100 and VlsE, besides the frequently detected p41 and OspC in the majority of EM cases.

Conclusion: These preliminary data suggest that the detection of active *Borrelia* infection by detecting ICs by PEG precipitation in the diagnostics of EM patients is not straightforward. Further research is necessary especially to investigate IC responses in patients with persistent symptoms.

P042

The MPN340 gene of *Mycoplasma pneumoniae* encodes a PcrA-like protein that lacks a 2B domain and displays bipolar DNA helicase activity

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Introduction: The DNA recombination and repair machinery of *Mycoplasma pneumoniae* is composed of a limited set of approximately 11 proteins. Two of these proteins were predicted to be encoded by the neighbouring open reading frames (ORFs) MPN340 and MPN341. Both ORFs were found to have sequence similarity with genes that encode proteins belonging to the DNA helicase superfamily 1 (SF1). Interestingly, while MPN341 is conserved between *M. pneumoniae* and its close genetic counterpart *Mycoplasma genitalium*, MPN340 is an *M. pneumoniae*-specific ORF that is not found in other mycoplasmas. Moreover, the length of MPN340 (1590 base pairs [bp]) is considerably shorter than that of MPN341 (2148 bp). A close inspection of the MPN340-encoded amino acid sequence further indicated that it may lack a so-called 2B subdomain, which is found in most SF1 DNA helicases. Also, the MPN340-encoded amino acid sequence was found to differ between subtype 1 strain M129 and subtype 2 strain FH at three amino acid positions.

Objective: The objective of this study was to elucidate the function of the proteins predicted to be encoded by the MPN340 ORFs from *M. pneunomiae* strains M129 and FH.

Methods: The MPN340 ORFs from strains M129 and FH were amplified by PCR and cloned into protein expression vectors. The MPN340-encoded proteins, which were named $PcrA1_{M129}$ and $PcrA1_{FH}$, respectively, were expressed in *Escherichia coli*, purified, and tested for their ability to interact with various DNA substrates.
Results: Both $PcrA1_{M129}$ and $PcrA1_{FH}$ were found to be divalent cation- and DNA-dependent ATPases that readily dissociated a wide variety of synthetic double-stranded DNA substrates into their complementary single strands. Although the proteins were also active on blunt-ended DNA substrates and substrates with 3' single-stranded extensions, they displayed highest DNA helicase activity on substrates carrying 5' single-stranded termini. Despite the differences between $PcrA1_{M129}$ and $PcrA1_{FH}$ in their amino acid sequences, the activities of these proteins were indistinguishable.
Conclusion: The *M. pneumoniae* proteins $PcrA1_{M129}$ and $PcrA1_{FH}$ are fully active SF1-like DNA helicases that lack a 2B domain and preferentially unwind DNA substrates that carry a 5' overhang.

P043

The RuvA homologues from *Mycoplasma genitalium* and *Mycoplasma pneumoniae* exhibit unique characteristics in DNA binding and in the interaction with RuvB and RecU

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Introduction: The DNA recombination and repair machineries of *Mycoplasma genitalium* and *Mycoplasma pneumoniae* differ considerably from those of gram-positive and gram-negative bacteria. Most notably, *M. pneumoniae* is unable to express a functional RecU Holliday junction (HJ) resolvase. In addition, the RuvB homologues from both *M. pneumoniae* and *M. genitalium* only exhibit DNA helicase but not HJ branch migration activity *in vitro*, in contrast to the RuvB protein from *Escherichia coli*. Another difference between the *Mycoplasma* RuvB proteins and the *E. coli* RuvB protein is that the activity of the latter could be stimulated significantly by another protein, i.e. RuvA. The role of the RuvA homologues from *M. pneumoniae* and *M. genitalium* is as yet unclear.
Objective: The objective of this study was to identify the function of the RuvA homologues from *M. pneumoniae* and *M. genitalium*.
Methods: The RuvA proteins from M. pneumoniae (RuvA*Mpn*) and *M. genitalium* (RuvA*Mge*) were expressed in *E. coli*, purified, and studied for their ability to bind DNA and to interact with RuvB and RecU.
Results: In spite of a high level of sequence conservation between RuvA*Mpn* and RuvA*Mge* (68.8% identity), substantial differences were found between these proteins in their activities. First, RuvA*Mge* was found to preferentially bind to HJs, whereas RuvA*Mpn* displayed similar affinities for both HJs and single-stranded DNA. Second, while RuvA*Mpn* is able to form two distinct complexes with HJs, RuvA*Mge* only produced a single HJ complex. Third, RuvA*Mge* stimulated the DNA helicase and ATPase activities of RuvB*Mge*, whereas RuvA*Mpn* did not augment RuvB activity. Finally, while both RuvA*Mge* and RecU*Mge* efficiently bind to HJs, they did not compete with each other for HJ binding, but formed stable complexes with HJs over a wide protein concentration range. This interaction, however, resulted in inhibition of the HJ resolution activity of RecU*Mge*.
Conclusions: The RuvA proteins from *M. pneumoniae* and *M. genitalium* have unique characteristics, not only in DNA-binding, but also in their interaction with other members of the DNA recombination and repair machinery.

P044

Molecular fingerprinting of *Candida parapsilosis* isolates in tracing bronchoscope contamination, from ITU patients, using automated repetitive Polymerase Chain Reaction

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Introduction: Nosocomial infections caused by *Candida parapsilosis* are often associated with the breakdown of infection control practices and/or the contamination of medical devices. Contaminated bronchoscopes have been linked to more outbreaks of healthcare-associated infections than any other medical device. Microbiological surveillance of bronchoscope reprocessing is appropriate to trace contamination of such devices and to prevent contamination and infection in patients after the procedures. Traditional automated identification and sensitivity systems for Candida species provide identification only at the genus/species level and do not possess any further discriminatory powers. There is a need for a simple, rapid and accurate method of identifying and typing Candida species within a hospital environment.
The aim of this study was to describe pseudo-outbreak of *C. parapsilosis*. Five ITU (Intensive Trauma Unit) patients were involved, one being immunocompromised. There were no clinical signs of disseminated candidiasis in the patients and no other respiratory samples other than broncheolalvoelar lavages (BALs) that were colonised with *C. parapsilosis*. The only commonality between the patients was that the BALs were taken by the same emergency bronchoscope.
Method: Ten isolates were identified on the VITEK® 2 system (BioMerieux) as *C. parapsilosis*. These isolates were

cultured on Sabouraud's Dextrose Agar with chloramphenicol (SABC) plates. Candida culture was extracted using the Mo Bio UltraClean™ Microbial DNA IsolationKit (Mo Bio Laboratories Inc.). Genomic DNA was extracted and 40-50ng of each sample was amplified using the DiversiLab® (DL) Candida Kit for DNA fingerprinting. The amplified products (1 µl) were separated and detected on the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) by using the DL repetitive sequence-based polymerase chain reaction (rep-PCR) system (BioMerieux). The results were analyzed by comparing the samples to the Candida rep-PCR DNA Fingerprint Library available with the DL software. The analysis utilized the DL software which uses Pearson's correlation coefficient for similarity calculation and rep-PCR patterns relationships were designated as recommended by the manufacturer.

Result: rep PCR analysis identified only two different patterns and two different groups. Nine out of 10 isolates were indistinguishable with > 99% similarity. One isolate was identified as a different pattern and fell in a different group with 65% similarity with the remaining isolates. Out of 10 isolates 6 were from patient's BAL samples and 4 isolates were from bronchoscope (two from saline flush and two from external swabs). The results proved that the bronchoscope was the source of the *C. parapsilosis* in the ITU patients.

Conclusion: As a result of this study the emergency bronchoscope was withdrawn from the use and sent back to the manufacture for further investigation. Root cause analysis of the incident resulted in the decontamination procedure of the bronchoscope being revised and improved standard operating procedures being introduced. The DiversiLab software which uses a standardized algorithm and automation allowed sample analysis, including a report to be completed for 13 samples in approximately 4 h. In conclusion, our study demonstrated the potential utility of automated rep-PCR and the DiversiLab system not only for disease outbreak investigations but also rapidly tracing and confirming contamination issues.

P045

Application of Multi Locus Sequence Typing to study the phylogenetic relationship between hypervirulent *Clostridium difficile* PRC ribotypes

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Introduction: *Clostridium difficile* is the most frequent cause of nosocomial diarrhoea worldwide. Since 2002, the reported incidence and severity of *C. difficile* infection has increased significantly, particularly of the notorious hypervirulent PCR ribotypes 027 (RT027) and RT078. Recently, PCR ribotypes that are closely related to RT 027 and RT 078 have been identified, representing a 027 lineage and a 078 lineage, respectively. However, little is known about their phylogenetic classification. Since Multi Locus Sequence Typing (MLST) is a technique suitable for studying the phylogenetic relationships, this technique was applied to analyze potential relationships of various *C. difficile* ribotypes within the 027 lineage and the 078 lineage.

Methods: 94 *C. difficile* isolates belonging to 75 different PCR ribotypes were typed using MLST. All *C. difficile* isolates have been collected at the National Reference Laboratory (RIVM, LUMC). MLST was performed as previously described by Griffiths et al., with slight adaptations. The concatenated MLST sequences of the seven housekeeping genes were used to construct a phylogenetic neighbour-joining tree with 1000 bootstrap resamplings using MAFFT analysis.

Results: The phylogenetic clearly demonstrates the clonal population structure of the *C. difficile* strains. In addition to the already described five evident clades of *C. difficile*, a potential sixth clade was discovered. This clade comprises of only one sequence type, ST-122 which corresponds to RT131. ST-122 is found in humans, although infrequently with a prevalence of 0.06%.

Furthermore, it was observed that the 027-lineage, consisting of ribotypes highly related to RT027, are all present within clade 2. The ribotypes of the 078-lineage were present within clade 5 and are all typed as ST-11, and are thus highly related. In this analysis it was found that ST-11 is highly divergent from the other sequence types, which was also confirmed by whole genome sequencing.

Conclusion: PCR ribotyping is a less effective characterisation method for studying close phylogenetic relations. In contrast, MLST can accurately link groups of isolates, as with the 027- and 078-lineages, and thereby appears to be an appropriate method for recognition and classification of potential hypervirulent strains. Furthermore, a new sixth clade is identified, of which the clinical and epidemiological relevance is under study.

P046

Microbial identification of vacteria using Matrix Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) in a routine microbiology laboratory

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Introduction: Matrix Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) has recently emerged as a new alternative for microbial

identification in the clinical microbiology laboratory. For accurate identification, a large database with a good algorithm that analyses the generated spectrum is crucial. In this study, microbial identification by MALDI-TOF MS in combination with the new Vitek MS database (bioMérieux), was compared with conventional biochemical and molecular methods in a routine microbiological laboratory.

Methods: A total of 447 isolates were evaluated in this study. Isolates comprised of Gram negative rods (n = 380), streptococci (n = 22) and staphylococci (n = 45). All isolates were identified by both MALDI-TOF MS (Vitek MS, bioMérieux) and conventional methods (Phoenix system, BD) according to the respective manufacturers' procedures. Congruent results between conventional and MALDI-TOF MS were considered definite. Discrepancies (genus or species) in identification were further analysed by molecular sequencing of the 16S genes. The result of 16S sequencing was considered the golden standard.

Results: Of the 447 isolates (53 different species from 27 different genera), 444 (99,3%) could be identified by the MALDI-TOF MS. Of these 444 isolates, 442 (98,9%) isolates were correctly identified to genus level and 439 (98,2%) isolates to species level by the MALDI-TOF MS.

Of the 3 isolates that could not be identified with the MALDI-TOF MS, 2 isolates were identified with 16S sequencing as *Bordetella avium* and *Bacillus massiliensis*. The third isolate was determined as *Acinetobacter lwoffii* by the Phoenix system.

The 3 isolates that were correctly identified only to the genus level were identified as *Neisseria weaveri* and *Enterobacter hormaechei* (2x) by 16S sequencing, but as *Neisseria subflava/animaloris* and *Enterobacter cloacae/asburiae* by the MALDI-TOF MS. Both *Neisseria weaveri* and *Enterobacter hormaechei* are not registered in the Vitek MS database.

The 2 isolates that were not correctly identified to the genus level were identified with 16S sequencing as *Massilia timonae* and *Empedobacter brevis*. The first isolate was identified as *Bordetella parapertussis* (and/or *Sutonella indologenes*) by the MALDI-TOF MS, *Massilia timonae* is not registered in the Vitek MS database. The *Empedobacter brevis* was identified as *Acinetobacter johnsonii* by the MALDI-TOF MS.

Conclusion: The results of the study show a high level of correct identification with MALDI-TOF MS of isolates that are commonly encountered in a routine clinical setting. The isolates that were not correctly identified by the MALDI-TOF MS are only sporadic found in the microbial laboratory. Misidentification might be due to the fact that not enough reference strains could be used for the construction of the database. Clinical relevance of these isolates can be argued and confirmation of the identification of these rare micro-organisms is subject to debate.

P047

Rapid change in the *Staphylococcus aureus* colonization patterns in patients with epidermolysis bullosa results in high immune responses against staphylococcal proteins

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Introduction: Patients with the genetic disease epidermolysis bullosa (EB) develop skin blisters upon minor injury. Depending on the type of EB, the symptoms vary in severity from minor blistering of the skin to a lethal form involving other organs. Because the natural barrier function of the skin is partially absent in patients with EB, wounds of these patients are colonized by microorganism, such as *Staphylococcus aureus*. This bacterium is a harmless commensal of about 11-32% of the human population. However, *S. aureus* can become invasive causing a range of infections including soft tissue infections and life-threatening systemic infections. It is known that the carrier status as well as the invasiveness of the staphylococcal disease has an influence on the antibody levels against *S. aureus*. In this study we focused on colonization by *S. aureus* over time in 35 patients with EB. Additionally, we determined the immune responses of 11 patients with EB against *S. aureus*.

Methods: 35 EB patients, divided into two groups: patients with chronic wounds (n = 12) and without chronic wounds (n = 23) were recruited. For each patient, swabs from the left nostril, right nostril, throat and three different wounds were taken 3 times with six monthly intervals, and the presence of *S. aureus* was tested with standard diagnostic tools. In total, we collected 443 *S. aureus* strains, which were analyzed by Multiple-locus Variable Number of Tandem Repeats Analysis (MLVA) and spa-typing. Additionally, whole blood samples were donated by 13 patients with EB to determine their immune responses against *S. aureus* by ELISA and Luminex technology.

Results: The typing experiments with the collected *S. aureus* strains revealed that 42% of the patients with chronic wounds carried only one MLVA type during all three sampling rounds. The other patients with chronic wounds (58%) were colonized over time by up to six different MLVA types. Only 4% of the patients without chronic wounds did not carry *S. aureus* during the period of sampling, whereas 43% of these patients carried one type and 52% were colonized by up to 5 different MLVA types. Comparison of immune responses against *S. aureus* cells showed that there is a significantly higher level of

anti-staphylococcal antibodies in patients with EB than in healthy carriers. Additionally, it was found that the median serum immunoglobulin G (IgG) levels against most of the measured staphylococcal virulence factors seem to be higher in the patients than in healthy controls.
Conclusions: Patients with EB are extensively colonized by *S. aureus* and frequently carry multiple strains over time. This results in elevated anti-staphylococcal antibody levels, which may be protective as judged by the low prevalence of staphylococcal diseases among this group of patients.

P048

Increased mupirocin use is associated with increased frequency of high-level mupirocin resistance in non-aureus staphylococci

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Background: In our hospital, mupirocin has increasingly been used for eradication of nasal *S. aureus* carriage in patients scheduled for certain surgical procedures over the past 6 years. The target for mupirocin is isoleucyl transfer RNA synthetase (*ileS*). High-level resistance to mupirocin is conferred by acquisition of plasmids expressing a distinct *ileS*-gene (*ileS-2*).
We aimed to assess the frequency of occurrence of high-level mupirocin resistance and look for a putative association with mupirocin use.
Methods: We assessed mupirocin resistance in non-aureus staphylococcal bloodstream isolates from 2006-2011, that had routinely been tested by Phoenix automated testing (PAT). In a sample survey, we evaluated reliability of PAT results using e-test in the first 40 consecutive non-aureus blood isolates of each year. PAT species determination was confirmed by maldi-TOF. We tested for presence of *ileS-2* in the first 100 consecutive non-aureus bloodstream isolates of each year using RT-PCR. Data on mupirocin use was acquired from Utrecht Patient Oriented Database.
Results: Sample survey (n = 237): Sensitivity and specificity of PAT to detect high-level mupirocin resistance was 0.97 and 0.97, respectively. Two isolates did not grow and PAT testing could not provide a result for one isolate e-test found. Thirty-two/237 (13.5%) of the isolates high-level mupirocin resistant. *IleS-2* RT-PCR was performed on 598 isolates. Eighty-five/598 (14.2%) of the isolates were phenotypically high-level mupirocin resistant. Phenotypical resistance agreed with presence of *ileS-2*, except for the following cases: in 4 phenotypically high-resistant isolates *ileS-2* RT-PCR was negative; 3 of these were *Rothia mucilaginosa*, *Kuceria* species, and *Micrococcus* species, known to be intrinsically resistant to mupirocin by other mechanisms than *ileS-2*. In 3 isolates *ileS-2* was detected (all with ct-values < 30), but were tested sensitive to mupirocin.
The yearly amount of mupirocin prescribed in our hospital increased from 3.6 kg in 2006 to 13.3 kg in 2010, and correlates with the increase in% non-aureus staphylococci carrying *ileS-2* (8% in 2006 to 22% in 2011; Spearman's rho 0.135, p = 0.01).
Conclusion: We observed a significant increase of high-level mupirocin resistant non-aureus *Staphylococci*, linked to presence of *ileS-2*. This increase coincides with an increased use of mupirocin.

P049

Influence of salt and pH on amoxicillin resistance in *Escherichia coli*

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Introduction: Due to the continuous increase of antibiotic resistance treatment of common infections is becoming more difficult and cost-intensive. Development of antibiotic resistant bacteria can occur as a result of mutation, conjugation or adaptation. Understanding the physiological response of antibiotic adapted bacteria can identify new drug targets or strategies for curbing the spread of resistant bacteria. In this study, the growth of an amoxicillin adapted *Escherichia coli* strain [1] was investigated at different sodium chloride concentrations and pH values. By comparing the maintenance energy of the antibiotic adapted and ancestor strain further physiological information was obtained.
Methods: *Escherichia coli* MG1655 was cultivated in a chemically defined minimal medium either supplemented with 0-3% sodium chloride or with an adjusted pH value ranging between 6 and 7,5. The growth of an *Escherichia coli* MG1655 strain that had been adapted by exposure to increasing amoxicillin concentrations was compared to the ancestor strain. Amoxicillin or tetracycline was added to the medium in varying concentrations according to the experimental design. The maximal specific growth rate and the maintenance energy were used as indicators for the comparison of the adapted and the ancestor strain. By measuring the specific glucose consumption in continuous cultures with a working volume of 250 ml and extrapolating to a dilution rate of 0 h^{-1} the maintenance energy of *Escherichia coli* wild type and the amoxicillin adapted strain was estimated.
Results: The maximum specific growth rate of the wild type and the amoxicillin adapted strain in the absence of antibiotics showed no difference. In the presence of amoxicillin increased salt concentrations revealed a stronger effect of the growth rate of the highly adapted strain compared to the ancestor strain. In contrast, the

influence of increasing salt concentrations on the growth rate was reduced by the addition of 1 µg/ml tetracycline for both strains. The determination of the growth rates using different pH values indicated a clear pH optimum for the wild type and adapted strain in the absence of antibiotics. The addition of amoxicillin resulted in an reduced pH influence for the antibiotic adapted strain. A distinct reduction of the pH effect on the growth rate was observed in the presence of tetracycline for both the wild type and the adapted strain. The maintenance energy did not differ between the antibiotic adapted and the ancestor strain. For both strains a maintenance energy of approximately 1 mmol g^{-1} h^{-1} was estimated.

Conclusion:

1.The antibiotic action of amoxicillin and tetracycline is affected by the sodium chloride concentration.

2.Tetracycline reduces the pH dependency of the growth rate of *Escherichia coli* wild type and the antibiotic adapted strain.

3. No difference in the maintenance energy between the antibiotic adapted and the ancestor strain was observed.

P050

Influence of primary tube handling on HIV-1 viral load quantification

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Background: With the introduction of more sensitive quantification methods of HIV-1 RNA, unexpected low-level viral loads ('blips") have been reported more frequently among strictly adherent patients on long-term cART.

At our laboratory we observed that these blips seemed to occur more often when long-term or crude transportation/ handling of samples had happened. We wanted to test the hypothesis that improper handling might induce "blips"in a standardized fashion.

Material and Methods: EDTA-blood was drawn in 6 PPT (K2-EDTA) tubes (Becton-Dickinson) from 15 HIV-1 infected individuals (10 with historically undetectable viral load, 5 with a detectable viral load) and 2 HIV-1 negative controls. After immediate centrifugation (10min 1500G), plasma was obtained immediately (0h) or after 6, 24, 48 or 72hrs. One tube was handled for 1h on a shaking tray (mimicking transport after centrifugation) before plasma was sampled. HIV-1 VL was quantified in the same run for all samples (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0, Roche)

Results: In 4 out of 10 patients with an undetectable viral load at t=0, a clear rise in viral load was observed after 1h shaking (range: 66-630 copies/ml). Samples from HIV-1 negative individuals had undetectable loads under all circumstances.

In samples from patients with a detectable viral load, after 72h a considerable drop in viral load was observed in 4 out of 5 patients (range 0.12 - 0.4 logs difference).

Conclusions: Improper handling of PPT tubes after centrifugation can lead to incorrect values of HIV-1 viral loads, risking to be interpreted as low level viremia.

These observations argue for stricter protocols for handling of blood samples to avoid the artificial rises in viral load.

P051

Hepatitis E virus genotype 3; an under-diagnosed pathogen causing chronic hepatitis in solid organ transplant recipients

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Introduction: Hepatitis E virus (HEV) has long been known as a major cause of acute hepatitis in developing countries, with occasional travel-related cases in developed countries. Travel-related HEV is mainly caused by genotype 1 and 2, strictly human viruses. Recently, HEV genotype 3 infection is being recognized as a public health issue in developed countries and is believed to be a porcine zoonosis. HEV was considered as an agent responsible for an acute hepatitis. Yet, increasing numbers of reports now show that HEV genotype 3 infection may lead to a chronic hepatitis in immuno-compromised patients.

Methods: The UMCG is a tertiary referral hospital providing care to both adults and children and has a large solid transplant program. Since the second half of 2007, HEV diagnostics is performed in patients treated in the UMCG with unexplained hepatitis. Serology is being performed with a HEV ELISA, followed by a confirmatory Western immunoblot. HEV RNA is being detected in plasma, serum or feces using a real-time PCR method. Subsequently, the HEV is characterized by sequencing of the ORF1 and ORF2 region. In a recent collaboration with the Medical University Vienna, HEV PCR was being performed in all the lungtransplant patients transplanted between 2009 to July 2011 showing ALT levels over 90 U/L at least once in the follow-up.

Results: In total, 32 patients were positive for HEV RNA in serum or plasma. The majority of the patients were immuno-compromised, 24 were solid organ transplant (SOT) patients and 5 were patients immunocompromised for other reasons. Only 3 patients diagnosed with HEV were immunocompetent. Chronic infection could be diagnosed in 19 of these SOT recipients (79%), 1 died of liver failure and 3 for other reasons. The time period between transplant and first positive sample ranged from

less than 1 year to 12 years (median 1 year). In total, 5 SOT recipients were able to clear the infection after lowering the immuno-suppressive medication and 6 were successfully treated with either pegylated interferon alpha-2b or ribavirin. Viral genotyping, strikingly, revealed only genotype 3 strains, all swine related.

Conclusion: HEV hepatitis is an under-diagnosed issue in immuno-compromised patients. Besides the general recommendation to lower the immuno-suppressive medication in these patients, there is currently no specific treatment option. However, both pegylated interferon alpha-2b and oral ribavirin have been described as potentially effective treatment of HEV infection. Early detection of HEV infection is essential to minimize liver damage and maximize the effect of antiviral therapy. Therefore, an increasing awareness for HEV among clinicians and medical microbiologists is needed.

P052

Investigation of the quantitative physiology of *Lactococcus lactis* at zero-growth state

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Introduction: In natural environments, due to variable-nutrients availability, microorganisms live in feast and famine existence, with famine as the prevalent state. When conditions are favourable, carbon and energy sources, are first consumed for growth-associated processes. Under conditions with limited nutrient supply, most metabolic energy is diverted to survival (maintenance) instead of growth. Many industrial fermentation processes also involve long periods of low availability of nutrients for microorganisms. An example is the process of cheese ripening, in which lactic acid bacteria in the cheese matrix are depleted from fermentable substrates. Survival under these conditions requires adaptations of cellular metabolism, and coincides with extremely slow or no-growth of the microorganisms. Zero-growth is defined as a metabolically active, non-growing state of a microorganism in which product-formation capability is maintained and thereby is fundamentally different from stationary phase or starvation.

Methods: Retentostat cultivation system has been designed to simulate zero-growth conditions. Retentostat cultivation is a modification of chemostat cultivation in which the growth-limiting carbon source is fed at a constant rate, while biomass is retained in the bioreactor by a retention filter-probe in the effluent line. Extended retentostat cultivation leads to growth rates that approximate zero while the rate of energy transduction equals the maintenance energy requirement. The aim of this project is to quantitatively examine zero-growth physiology of the plant-derived lactic acid bacterium, *Lactococcus lactis*.

Results: After cultivation of *L. lactis* at extremely low growth rates in glucose-limited retentostat conditions, cell physiology, metabolic profile, and robustness of the strain were investigated. Moreover, growth kinetic parameters, substrate- and energy-related maintenance coefficients and biomass yields were calculated from the retentostat cultures. Specific growth rates decreased to 0.0001 h^{-1} after 42 days, while doubling times increased to over 260 days for two parallel cultures. In addition to this, viability of the overall culture was> 95%, as assayed with the LIVE/DEAD *BacLight* kit using FACS. While both fermentations displayed very similar end-product profiles, there were two metabolic shifts between homo-lactic and mix-acid fermentation patterns during the retentostat cultivation. The biomass concentrations were accurately predicted by a maintenance coefficient of 1.1 mmol of carbon g^{-1} of biomass h^{-1}calculated from retentostats.

Conclusions:

1. Adaptation of *L. lactis* to zero-growth state, which is principally different than carbon starvation, under limited carbon source was investigated at metabolome level.
2. The transition from a growing to a non-growing state under retentostat conditions is progressive, in contrast to sudden transition in batch cultures.
3. During retentostat conditions, metabolic behavior of *L. lactis* remarkably fluctuated between homo- and hetero-fermentative patterns.
4. Biomass accumulation and specific rates of glucose consumption at zero-growth rates could be accurately predicted based on a maintenance coefficient calculated from the retentostat cultures.

P053

Poultry-associated ESBL-positive *E. coli* grow in an ex vivo gut flora system without antibiotic pressure

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Introduction: We recently showed that 94% of Dutch retail poultry meat samples contained ESBL producing isolates of which 39% belonged to *E. coli* genotypes also present in human samples and located on IncI1 plasmids that were genetically indistinguishable from those obtained from poultry (meat). Based on these data we hypothesized that transmission of ESBL genes, plasmids and *E. coli* isolates from poultry to humans, most likely through the food chain, does occur.

To gain further support for this hypothesis we determined in this study whether ESBL-positive *E. coli* isolated from poultry (meat) grow in a model mimicking the human gut flora, and if so, whether horizontal

transfer of ESBL-positive plasmids to the human gut flora may occur.

Methods: A faeces culture of six healthy individuals was blended and used as representative for the gut microbiome. Nine *E. coli* strains with ESBL-positive plasmids found in both poultry (n = 5) and humans (n = 4) were selected. Resistance to rifampicin was introduced by growing the strains overnight in LB medium with progressively higher concentrations of rifampicin. Compensatory mutations were allowed to occur by extending the growth for 48 hours. Rifampicin specific mutations were confirmed by PCR and sequencing of the rpoB gene.

The representative faeces culture was added to diluted SIEM (diluted Standard Ileal Efflux Medium) containing a strong buffer and grown under anaerobic conditions. The strains were normalized to a standard number of CFUs using OD measurement. A dilution series was made, which was used to spike the culture in SIEM. Growth was determined by plating on Violet Red Bile Dextrose Agar (VRBD) plates containing 1 µg/ml cefotaxime and the number of resistant *E. coli* in the spiked culture was calculated. The plates were copied to VRBD plates containing 1 µg/ml cefotaxime and 25 µg/ml rifampicin. All colonies that did not emerge on the second plate were tested for the presence of plasmid and beta-lactamase.

Results: All strains were able to establish themselves in the flora system and showed growth with an average of 2 log 10. The required spike could be as little as 100 CFU. No difference in *E. coli* obtained from poultry or humans was observed. PCR and plasmid based replicon typing of the colonies that did not emerge on the second plates are ongoing to confirm the possible transfer of the ESBL gene or plasmid.

Conclusion: *E. coli* associated with poultry and expressing PA-ESBL can grow in the presence of human flora even without the selective pressure of antibiotics. The number of CFU required are within the lower limit of the CFU range found on chicken meat. This is in agreement with the hypothesis that poultry may contribute to the spread of ESBLs among the human population.

P054

Immunosenescence contributes to increased pneumococcal colonization in aged mice

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Introduction: Nasopharyngeal colonization with *Streptococcus pneumoniae* is usually asymptomatic, though an important prerequisite for disease. Invasive pneumococcal disease disproportionably affects the very young and the elderly; the factors behind the increased risk for the elderly are poorly understood. The aim of this project was to study potential mechanisms of increased risk of pneumococcal infection using an elderly mouse colonization model and focusing on the innate host response.

Methods: Female C57Bl/6 mice aged 18-23 months (elderly) and 3-4 months (young controls) were intranasally inoculated with serotype 6B pneumococci. Density and duration of colonization were determined 1-2 times a week for 4 weeks. In addition, we investigated dynamics of immune cells infiltrating into the nasopharynx by flow cytometry. Cell signalling in the nasopharyngeal associated lymphoid tissue (NALT) was studied using quantitative PCR.

Results: Although there was no significant difference in colonization density between elderly and young mice at day 3, elderly were colonized significantly longer. We observed greater immune cell numbers in the nasopharynx of elderly before colonization plus a larger increase in cell influx in the days post-inoculation compared to young controls. In young mice clearance of bacteria at day 7 strongly correlated with an influx of monocytes/macrophages. These dynamics were less apparent in elderly mice. Also, young mice significantly increased expression of IL-1b and NLRp3 after colonization, while elderly did not. Transcript levels of most other genes tested in elderly NALTs were equal to or greater than in young NALTs.

Conclusion: Elderly mice were not able to clear pneumococcal colonization as quickly as controls. Whether this process is causally related to the observed dynamics in monocytes/macrophages needs further study. Furthermore, no defect in production of innate immune related mRNA transcripts in NALT has yet been observed.

P055

Prospective study on prevalence and characteristics of ESBL/AmpC producing *Escherichia coli* isolated from veal calves at slaughter

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Introduction: In the Dutch veal calf industry the majority of animals are imported from many different European countries and are subsequently piled into new herds on veal farms. As a result, on each of these farms, all animals originate from different dairy farms. Therefore these farms act as a melting pot for antimicrobial resistant bacteria and their genes. Extended spectrum beta lactamase (ESBL)/AmpC producing *Escherichia coli* have been

reported to occur in many food animal species in different frequencies. The focus of this study was to determine the prevalence and molecular characteristics of plasmid mediated ESBL/AmpC's in *E. coli* isolated from veal calves at slaughter.

Methods: As part of an ongoing study in 2011 in 100 slaughter batches originating from different farms, 10 veal calves per batch were screened for the presence of plasmid mediated ESBL/AmpC-producing *E. coli*. So far, 83 flocks are sampled. Faecal samples were collected from 10 individual calves in each farm by rectal swabs. The faecal samples were cultured in LB enrichment broth supplemented with 1 mg/L cefotaxime and incubated over night at 37°C. Subsequently, a MacConkey agar plate supplemented with 1 mg/L cefotaxime was inoculated with the culture from the enrichment broth. The MacConkey agar plates were incubated over night at 37°C. From each flock one isolate was selected for molecular characterization of ESBL/AmpC genes. Chromosomally encoded AmpC's were not taken into account. These isolates were characterized using disk diffusion assays, Identibac AMR-ve micro array, PCR- and sequence analysis.

Results: In 66% of the slaughter batches, one or more faecal samples harboured *E. coli* producing ESBLs. Genotypic analysis showed that *bla*CTX-M-1, -2, -14, -15, -32 and *bla*TEM-52 enzymes were present. In approximately 50% of the isolates harbouring either *bla*CTX-M-1 or -15, the isolate also harboured *bla*OXA-1. So far, no other plasmid mediated ESBL/AmpC's were detected. From 4 of the 83 farms sampled so far, less than 10 individual calves were sampled. These were still included in the study.

Conclusion: This study showed that at slaughter 66% of the veal calf slaughter batches were positive for ESBL producing *E. coli*. These were mainly *bla*CTX-M variants. Next to *bla*CTX-M variants and *bla*TEM-52, no other plasmid mediated ESBL/AmpC's were detected. Molecular characterization of genes, strains and plasmids to determine genetic relatedness is pending.

P056

E. coli from Dutch patients and retail chicken meat share the same plasmid encoded AmpC gene and plasmids

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Introduction: We recently showed that 94% of Dutch retail poultry meat samples contained ESBL producing isolates of which 39% belonged to *E. coli* genotypes also present in human samples and 19% of a representative sample of Dutch clinical ESBL positive *E. coli* isolates contained ESBL genes located on IncI1 plasmids that were genetically indistinguishable from those obtained from poultry (meat). The data suggested the transmission of ESBL genes, plasmids and *E. coli* isolates from poultry to humans, most likely through the food chain.

The aim of this study was to determine whether poultry meat may next to ESBLs also be a reservoir of plasmid encoded AmpC genes (pAmpC) and if so in which proportion of the Dutch clinical isolates.

Methods: In 2009 479 ESBL-screen positive human clinical *E. coli* isolates from 31 laboratories all over the Netherlands were collected. In this collection 102 *E. coli* showed a minimal inhibitory concentration of> 1 mg/L for cefotaxime or ceftazidime and> 16mg/L for cefoxitin in broth microdilution (Merkel Diagnostic GmbH, Rüsselsheim, Germany) according to the CLSI guidelines. These isolates were included in this study.

In 2010 163 ESBL suspected isolates were collected from 98 chicken retail meat samples. Twelve isolates suspected of AmpC production on the basis of their phenotype were screened for AmpC production in by using PM/PML Etests (BioMérieux, Marcy L'etoile, Lyon, France).

A multiplex PCR designed for pAmpC's and sequencing was performed for the detection of CMY-2/-55. Thirty available isolates (88%) were selected for plasmid based replicon typing (PBRT). PBRT was performed and selective plates, substituted with 1 µg/ml cefotaxime and clavulanic acid, were used to screen for isolates carrying AmpC. PCR and sequencing was performed to confirm the presence of CMY-2/-55 and detect the presence of any other possible β-lactamase on the same plasmid.

Results: Twenty-two of 102 human clinical *E. coli* isolates suspected of AmpC production and 12 chicken retail meat *E. coli* isolates cultured from 11 (11%) meat samples harboured blaCMY-2/-55. Eight of 19 clinical human isolates (43%) harboured CMY-2/-55 on an IncK plasmid, 9 human isolates (47%) harboured CMY-2/-55 on an IncI1 plasmid, and two isolates harboured the pAmpC on a untypeable plasmids. None of the plasmids carried another beta-lactamase.

Eleven unique chicken retail meat samples were selected for further investigation. Ten of eleven chicken retail meat isolates (91%) harboured CMY-2/-55 on an IncK plasmid and one isolate (9%) harboured CMY-2/-55 on an IncI1 plasmid. One chicken retail meat sample, containing the IncI-type plasmid, harboured also a CTX-M-1 group ESBL on the same plasmid.

Conclusion: Human clinical and chicken retail meat isolates share the same combination of CMY-2/-55 β-lactamase and rare IncK plasmid, supporting the hypothesis that chicken retail meat may act as a possible reservoir for beta-lactamases.

P057

Correct identification of *Haemophilus influenzae* and *Haemophilus haemolyticus* by matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry

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Objective: Generally accepted laboratory methods that have been used for decades do not reliably distinguish strains of H. haemolyticus from strains of H. influenzae. Misidentification of commensal H. haemolyticus as pathogenic H. influenzae may result in unnecessary use of antimicrobials. To distinguish H. influenzae from H. haemolyticus we created a new database on the MALDI-TOF Bio-typer 2 and compared MALDI typing with routine bacteriological and molecular methods, including multilocus sequence analysis.

Materials and methods: The bacterial isolates (n = 33) were identified on the basis of the growth requirement for haemin (X-factor) and nicotinamide adenine dinucleotide (V-factor). The used strains were selected, for biodiversity, on the basis of the results of Slide Agglutination Sero Typing and included 5 capsulated and 28 uncapsulated strains. We created a new database containing reference strains ATCC 49766 (HI), ATCC 33390 (HH) and an in-house reference strain. The results were compared with MLST and species identification by using ompP6 PCR. For cross-identification and spectra quality control all strains were also tested by the Bruker Daltonics Laboratory.

Results: Based on colony morphology and the requirements of X- and V-factor all 33 strains were identified as H. influenzae. Determination by using MALDI Biotyper 2, 14 (42%) of the 33 strains were identified as H. influenzae and 19 (58%) as H. haemolyticus. MLST and species identification using the ompP6 gene gave 100% agreement when compared with mass spectrometry identification. The same results were obtained at the Bruker laboratory using the identical set of micro-organisms. In a MSP dendrogram the strains of the two species cluster separately, this separation also was supported by Principle Component Analysis.

Conclusion: The identification of H. influenzae and H. haemolyticus based on conventional method is not reliable. MALDI-TOF mass spectrometry is a reliable and rapid technique for distinguishing HI and HH and comparable with molecular techniques. Accurate identification of pathogenic H. influenzae is important and may contribute to a reduction in unnecessary antibiotic used for the treatment of misidentified H. influenzae.

P058

Consequences of non-compliance in treatment of murine tuberculosis caused by the Beijing genotype on therapy efficacy and emergence of resistance

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Introduction: Resistance to anti-tuberculosis (TB) drugs is strongly increasing worldwide. According to the Global Report of the WHO, already nearly half a million cases of multi-drug resistant TB (MDR-TB) are recorded annually. Moreover, in almost all countries also extensively-drug resistant TB (XDR-TB) has been reported. Because only a minority of the MDR-TB cases can currently be treated according to the WHO guidelines, resistant TB may increasingly become an untreatable disease.

Non-compliance is considered one of the factors contributing to development of resistance to anti-TB drugs. A recent observation related to resistance is that particular strains seem more prone to develop MDR-TB than others. For instance, Beijing genotype strains have been associated with MDR-TB and XDR-TB, treatment failures and relapse after treatment. The East-African-Indian (EAI) genotype strains are negatively associated with these problems.

The aim of the present study in our established mouse TB model is to investigate whether the consequences of suboptimal treatment, simulating non-compliance in humans, would be more pronounced in the outcome of TB caused by the Beijing genotype in comparison to TB caused by the EAI genotype.

Methods: BALB/c mice were infected either with a *Mycobacterium tuberculosis* (Mtb) Beijing or EAI strain through intratracheal inoculation followed by inhalation. The mice were treated with dosage and schedules of anti-TB drugs derived from current clinical guidelines with a treatment duration of 26 weeks (compliance group). In the non-compliance groups drugs were administered for duration of only 13 weeks and additionally in a reduced treatment frequency. These mice received treatment either five days per week (non-compliance group A) or three days per week (non-compliance group B) or once a week (non-compliance group C). At various time points the mycobacterial load in infected organs was assessed, cytokine- / chemokine levels in blood were determined and histopathological examination was performed.

Results: The therapy efficacy in the treatment compliance and treatment non-compliance groups was similar for the Beijing-infected mice compared to the EAI-infected mice. Assessment of relapse after an additional 13 weeks

post-treatment period revealed no relapses of TB in the compliance condition. Under the non-compliance conditions Beijing-infected mice as well as EAI-infected mice showed relapse of TB, as assessed 13 weeks after termination of treatment. However, only in Beijing-infected mice isoniazid-resistant mutants were selected at 5 and 13 weeks post-treatment in non-compliance group C. The minimal inhibitory concentration of isoniazid for the isoniazid-resistant mutants was 128 mg/L. No changes in the *katG, inhA, ahpC or kasA* genes were found.

Conclusion: Both Beijing strain-induced TB and EAI strain-induced TB in mice is treatable if a proper anti-TB treatment regimen is used. Non-compliance by short treatment duration and low treatment frequency resulted in regrowth of Mtb and thus relapse of infection.

Beijing genotype strains are more prone to develop isoniazid resistance than strains of the EAI genotype, which may contribute to the worldwide expansion of TB caused by the Beijing genotype and the emergence of resistance.

P059

Prevalence of post-operative wound infections after abdominal and vascular surgery, during a period of 3,5 year

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Introduction: The effect that post-operative wound infections (POWIs) have on patient safety depends in part on the type of infection, i.e. superficial, deep or organ/space. Since a decrease in hospitalization period after surgery becomes common practice, it is to be expected that an increased number of POWI will be diagnosed after discharge. As POWIs negatively influence patient safety, hospitals in the Netherlands have to participate in a national programme, i.e. the VMS safety programme ("veiligheidsmanagementsysteem"), aiming to improve the adherence of the infection control policy, to reduce the prevalence of POWI and to improve patient safety. The objective of this study was to determine the effect of the VMS safety programme on the prevalence of POWIs during and after hospitalization among surgical patients.

Methods: We assessed the prevalence of POWIs from July 2008 until December 2011, according to the criteria of the Centers for Disease Control and Prevention (CDC). We differentiated between the prevalence of POWI during hospitalization and after discharge, between superficial, deep and organ/space infection, and between patients who were treated exclusively in our hospital and patients referred from another hospital. The POWI prevalence during hospitalization in relation to the wound class was assessed. Data were examined for abdominal and vascular index-surgeries only, during a 30 days follow-up period after surgery.

Results: 2061 abdominal and 1645 vascular surgical procedures were included. The overall prevalence of POWI after abdominal surgery was 22%, of which 49% was superficial, 45% deep and 6% organ/space. Of all abdominal infections 27% were found after discharge. After vascular surgery the figures were 8,5%, of which 54% was superficial, 39% deep and 7% organ/space and 43% were found after discharge.

Excluding the POWIs among referred patients from the analysis, the overall prevalence of POWI after abdominal surgery was 14%, 29% of which were found after discharge. After vascular surgery the figures were 5% and 44% respectively.

The prevalence of POWI in relation to woundclass for abdominal surgery was as follows: 2,8% for clean wounds, 16,8% for clean-contaminated wounds, 16,4% for contaminated wounds and 22,0% for dirty wounds. For vascular surgery the infection prevalence for the four wound classifications were 2,8%, 3,6%, 12,3% and 18,2% respectively. We observed only a decreasing trend in the contaminated wounds of abdominal POWIs and the clean-contaminated wounds of vascular POWIs after implementation of the VMS programme.

Conclusions: The VMS safety programme did not show a significant reduction of POWI in patients undergoing abdominal or vascular surgery. Large differences were found in POWI prevalence between the two surgical disciplines. The follow-up of patients after hospital discharge and the patients referred to our hospital contributed in a higher infection prevalence. Other related factors that influence the revalence of a POWI need to be further investigated.

P060

The usefulness of various typing methods for long term epidemiology of MRSA

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Introduction: Since 2008, several new unexpected MRSA cases, without any known risk factors, were found in a Dutch hospital. Spa-typing revealed 3 closely related Spa-types; t015, t589 and t1331. These types are rarely found in other parts of the Netherlands (www.rivm.nl/mrsa).

A total of 21 patients and Healthcare Workers (HCWs) were involved; one outbreak occurred on the surgical

unit. Eleven patients and 2 HCWs were found during the outbreak. Seven patients were admitted on surgical units or to the ICU without any epidemiologically linkage and were probably acquired in the community. A third HCW was found during contact tracing of a patient not related to the outbreak. Recently a patient (Pat-X) was admitted to the surgical unit who carried an MRSA with a similar Spa-type (t015), which could indicate ongoing transmission as part of the outbreak. Additional typing methods were employed to get a better understanding of the epidemiology.

Methods: Amplified Fragment Length Polymorphism (AFLP) was performed on all isolates in the VU University Medical Center. Multiple-Locus Variable number tandem repeat Analysis (MLVA) was performed at the National Institute for Public Health and the Environment (RIVM) performed the MLVA typing. Raman spectroscopy was performed at the Amphia hospital.

Results: With the exception of Pat-X, all patients and HCWs had related strains using AFLP, MLVA and Raman spectroscopy.

Conclusion: During 3 years, 7 patients with related MRSA strains with a likely origin in the community were found. This strain caused an outbreak in the hospital involving 11 patients and 2 HCWs. Also one HCW was found related to an individual patient. A recently admitted case with a similar Spa-type, who was suspected to have acquired the strain in the hospital, turned out to be unrelated using AFLP, MLVA and Raman. The recently observed changes in the epidemiology of MRSA complicate the work up of outbreaks and may require additional typing methods.

P061

Antimicrobial resistance of *Staphylococcus aureus* isolates from primary care patients and nursing home residents in the Netherlands and Germany

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Objectives: We assessed the antibiotic resistance of *Staphylococcus aureus* isolated from primary care (GP) patients and nursing home (NH) residents in the Netherlands and Germany in the Euregion Meuse-Rhine. Actual data of antibiotic resistance of commensal isolates are essential for an optimal empiric choice.

Methods: A total of 315 and 200 nasal swabs were collected from Dutch and German GP patients, respectively. 356 and 343 nasal swabs were collected from Dutch and German NH residents. Susceptibility testing was performed with the micro broth dilution method according to EUCAST guidelines. Putative meticillin resistant *S. aureus* (MRSA) isolates were confirmed for the presence of *mecA* with pcr. Determination of the *spa* locus, Staphylococcal cassette chromosome (SCC)*mec* typing, pulsed-field gel electrophoresis and multi locus sequence typing (MLST) was performed on all MRSA isolates.

Results: Prevalence of *S. aureus* carriage was 32% among the Dutch participants (n = 98 from Dutch GP patients and n = 121 from Dutch NH residents) and 26% and 39% among the German GP patients (n = 76) and NH residents (n = 136), respectively.

Significant differences in antibiotic resistance between the German NH isolates and the other groups of isolates were found for oxacillin ($p < 0.001$), the macrolides ($p < 0.001$), ciprofloxacin ($p < 0.001$) and gentamicin ($p < 0.005$). Point of concern was the high resistance to ciprofloxacin among the Dutch (23%) ($p < 0.001$) and German (43%) ($p < 0.001$) NH isolates compared with those from the GP (0% and 3%). Overall resistance to fusidic acid, mupirocin, tetracycline, gentamicin and daptomycin was less than 5%, whereas resistance to linezolid, trimethoprim-sulfamethoxazole and vancomycin was not observed

MRSA prevalence was 6% among the German NH residents (n = 19), and was less than 1% in the other groups. Among the MRSA isolates from the German NHs two clones were demonstrated i.e. ST225, SCC*mec*II with *spa* type t003 and t151 (n = 15) and ST22, SCC*mec*IV with *spa* type t032 (n = 4). Both are hospital associated MRSA clones.

Conclusions:

1. Resistance to oxacillin and the macrolides of *S. aureus* was relatively high among German NH isolates as was the prevalence of MRSA compared with the other groups of isolates.
2. The resistance to ciprofloxacin was high among NH isolates both in the Netherlands and Germany.
3. Among the German NH residents two MRSA clones were demonstrated, which are globally spread hospital associated MRSA clones.
4. The differences in resistance between Germany and the Netherlands are very likely due to differences in antibiotic use and infection control policies.

P062

Susceptibility of routine methicillin-resistant *Staphylococcus aureus* isolates to a series of antibiotics in the Netherlands

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Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen that is associated with serious infections. In the Netherlands, the incidence of MRSA infections remains low due to the restricted use of antibiotics and the implementation of an active search and destroy policy. Although the Netherlands has a successful surveillance system for typing MRSA, very little is known about the incidence of resistance to antibiotics other than methicillin in these isolates. Therefore, susceptibility to a broad spectrum of

antibiotics was tested for randomly chosen MRSA isolates to investigate the susceptibility of these isolates and the results were compared with our typing data.

Methods: A total of 246 MRSA isolates from our national MRSA surveillance were used in this study. All isolates were typed with multiple-locus variable number of tandem repeat analysis (MLVA). Susceptibility was tested for: cefuroxime, ciprofloxacin, clindamycin, daptomycin, erythromycin, gentamicin, linezolid, meropenem, mupirocin, quinupristin-dalfopristin, rifampicin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole and vancomycin. The isolates were tested according to the EUCAST criteria.

Results: All 246 isolates were resistant to at least one antibiotic and resistance was found to all antibiotics with the exception of tigecycline and vancomycin. Most isolates (76, 31%) were resistant to 4 antibiotics. Six isolates (2%) showed the broadest resistance (8 antibiotics). Most resistance was found to gentamicin (240 isolates, 98%) and cefuroxime (219, 89%). Resistance was also found to erythromycin (119, 48%), tetracycline (111, 45%), ciprofloxacin (95, 39%), clindamycin (66, 27%), meropenem (27, 11%), mupirocin (18, 7%), trimethoprim/sulfamethoxazole (9, 4%), daptomycin (8, 3%), linezolid (2, 1%), rifampicin (2, 1%) and quinupristin-dalfopristin (1, 0.5%).

MLVA-typing resulted in 243 complete MLVA-profiles. MLVA complex (MC)398 was predominant with 78 (32%) isolates, followed by MC5 (38, 16%) and MC8 (32, 13%). Three clear correlations were found between MLVA-typing results and the presence or absence of antibiotic resistance. Firstly, of the 15 isolates with MC22, 12 (80%) were meropenem resistant. Secondly, tetracycline resistance was predominant within MC398 isolates (76, 68%). Finally, we found a noticeable absence of clindamycin and erythromycin resistance within isolates belonging to MC45.

Conclusion: Our results show that most MRSA isolates are resistant to 4 or more antibiotics and resistance levels to some antibiotics were 89% or higher. No resistance was found to tigecycline and vancomycin. Molecular typing of all isolates revealed that the presence or absence of resistance to some antibiotics could be linked to specific MLVA complexes. These results show that, despite the restricted use of antibiotics, antibiotic resistance among *S. aureus* isolates is widely present in the Netherlands and a good surveillance system is necessary to monitor possible changes in antibiotic resistance in the future.

P063

The occurrence of ESBL/AmpC-producing *E. coli* and MRSA in drinking-water on pig and broiler farms

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Introduction: Not much is known about the role of drinking-water in the spread of antimicrobial resistance between animals within a farm. Therefore, a study was conducted to measure the microbial quality of drinking-water samples on pig and broiler farms. As part of this study samples were screened for the presence of MRSA and ESBL/AmpC-producing *E. coli*.

Methods: The study was performed on a selection of twenty-nine broiler farms and thirty-three pig farms (sows and fattening pigs) distributed throughout the Netherlands. On each farm a technical inspection of the drinking-water system was performed by Kewi Services who also organized the sampling of the drinking-water system. Samples were taken from different parts of the drinking-water system including water pipes and drinking troughs. Besides, material was taken from the inside of water pipes with dry cotton swabs. From each farm samples were sent to the Central Veterinary Institute-Lelystad of Wageningen UR (CVI) for isolation of MRSA and ESBL/AmpC-producing *E. coli*.

At CVI volumes of 500 ml of all samples were filtered through 0.45 µm cellulose membranes. These membranes and the swabs were used for culturing procedures. Culturing of MRSA was performed with pre-enrichment in Mueller Hinton broth + 6.5% NaCl followed by selective enrichment in Phenol Red Mannitol Broth + 5 mg/L ceftizoxim en 75 mg/L aztreonam and culturing on MRSA Brilliance Screen agar. Typical blue colonies were confirmed with PCR.

Culturing of ESBL/AmpC-producing *E. coli* was performed with selective enrichment in Luria-Bertani + 1 mg/L cefotaxime followed by culturing on MacConkey agar + 1 mg/L cefotaxime. Typical pink colonies were tested for indole production. To determine the ESBL phenotype disk diffusion tests were performed on Mueller Hinton agar. The ESBL or AmpC-genes were identified with different simplex PCR's targeted at bla_{TEM}, bla_{CTX-M}, bla_{SHV} and bla_{CMY} followed by sequencing of the amplicons.

Results: A total number of 255 samples from 62 farms were tested either for the presence of MRSA or ESBL/AmpC-producing *E. coli*. As a result 35 MRSA isolates were identified in 22 farms, mainly in pig farms (sows and fattening pigs). Twenty ESBL or AmpC-producing *E. coli* were detected in 16 farms, equally spread over pig and broiler farms. Sequencing of the amplicons is pending, however PCR revealed the presence of *E. coli* isolates with bla_{CTX-M} (n = 6), bla_{CTX-M} and bla_{TEM} (n =2), bla_{TEM} (n = 8), bla*CMY* (n = 2) and bla_{CMY} and bla_{TEM} (n = 1). In one isolate no ESBL or AmpC genes were detected.

Conclusion: The majority of the MRSA isolates were found in drinking-water samples in pig farms. However, MRSA was also isolated in two broiler farms. ESBL/AmpC-producing *E. coli* were found in drinking-water samples from both pig and broiler farms.

These results demonstrate that the drinking-water system can be a source of transmission of multidrug resistant organisms on pig and broiler farms.

P064

Recombinant bacillus Calmette-Guérin as an antigen carrier for cancer immunotherapy

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Introduction: Bacillus Calmette-Guérin (BCG) is a live attenuated vaccine currently used to combat tuberculosis. Apart from this specific protection BCG is also known as a particularly strong adjuvans and is able to break immunological tolerance against antigens.
Research on the immunologically beneficial effects of BCG to reduce tumor size in cancer has had varied results. Beneficial effects on other tumor types have been observed in multiple studies using recombinant BCG (rBCG) expressing tumor antigens and/or recombinant cytokines. The heterologous antigens or cytokines are usually fused to BCG signal sequences targeting the antigens to either the surface of the bacterium or in soluble form to the extracellular environment. Research has suggested that localization of antigens can have a big impact on the level and type of T-cell responses. However, influences of antigen expression level, localization and secretion efficiency have not been studied very thoroughly and a better understanding of these factors might lead to a vaccine, usable against tumors e.g. acute myeloid leukaemia (AML). Our aim therefore as a microbiological group, is to use alternative cloning strategies to accomplish higher expression levels and more efficient and better defined secretion of heterologous proteins. Hereby we aim to increase the tumorantigen specific immune responses which will be analysed in cell culture assays.
Methods: Signal sequences were amplified from Mycobacterium tuberculosis (H37Rv) DNA using PCR. The human G-CSF gene was amplified from the plasmid pORF-hGCSFb (purchased from Invivogen, CA, USA). Plasmid LS7 containing MART-1 cDNA was kindly supplied by Dr. Erik Hooijberg (VUmc, CCA). Optimized MART-1 was ordered from Geneart (Life technologies, Paisley, United Kingdom)
Chimeric genes cloned into the pSMT3 shuttle vector and electroporated into M. marinum strain E11. 0.5% Genapol detergens was used to extract cell surface fractions. Proteins were visualized using western blot and antibody staining with either anti-human Melan-A clone A103 (Monosan), or rabbit polyclonal to G-CSF (Abcam, Cambridge, United Kingdom)
Results: In M. marinum we have achieved heterologous expression and highly efficient secretion of the human cytokine granulocyte colony stimulating factor (G-CCF) by fusing the g-csf gene to a Type-VII secreted protein. This chimerical protein was visualized by immunoblot using rabbit anti-hGCSF. The secreted fusion protein remains attached to the cell surface of M. marinum as determined by detergent surface extraction, it was not found in the culture supernatant and < 10% was found in detergent treated cells. Additionally we have found that codon optimization and other modifications in the human tumor associated antigen (TAA) Melanoma antigen regognized by T-cells (MART-1) does not lead to higher expression level, but does increase the stability of chimerical fusions of mycobacterial signal sequences to MART-1.
Concusion: We have shown an alternative method of transporting heterologous proteins to the mycobacterial cell surface, as well as a method to increase chimerical protein stability.

P065

The prevalence and antibiotic susceptibility of *Staphylococcus aureus* isolated from general practice patients in several European countries

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Introduction: Over 90% of all antibiotics in Europe are prescribed in the outpatient setting. Despite this fact, most primary care guidelines are based on hospital data. The APRES study (acronym for 'Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance') wants to fill this gap regarding infections, among general practice (GP) patients, caused by *Staphylococcus aureus* and *Streptococcus pneumoniae* in nine countries, i.e. Austria, Belgium, Croatia, France, Hungary, Spain, Sweden, the Netherlands and the United Kingdom. This will be done by determining the antibiotic susceptibility of *S. aureus* and *S. pneumoniae* strains, isolated from GP patients in these countries, and relate these susceptibilities to antibiotic prescription data from electronic medical records of the participating GPs. Here, we report the prevalence rates of *S. aureus* strains on a country and general practice level. The antibiotic susceptibility of *S. aureus* from the Netherlands will be compared with those from several other participating countries.

Methods: From November 2010 to August 2011, general practices (n = 20 per country), recruited through existing GP networks, collected nose swabs and relevant patient information from patients (n = 200 per general practice) visiting their practice for a non-infectious reason. The main inclusion criteria were: no hospitalisation or antibiotic use in the previous 3 months and aged 4 years and older (in the UK 18 years and older due to ethical reasons). Nose swabs were first sent to a national laboratory for identification of *S. aureus* using standardized methods and subsequently to the central microbiological laboratory of Maastricht University Medical Centre for susceptibility testing. Putative methicillin-resistant *S. aureus* (MRSA) will be confirmed by PCR.

Results: In total 32,630 nose swabs were collected, and in 21.4% (n = 6985) of these swabs a *S. aureus* strain could be isolated. The highest prevalence was observed in Sweden and the Netherlands (29.4% and 27.7%, respectively), whereas the lowest prevalence was seen in Hungary and Austria (14.2% and 16.8%, respectively). In all nine countries, large differences were observed on the practice level. The largest difference in *S. aureus* prevalence per practice was observed in Croatia (range: 12.4% to 31.9%), and the lowest difference in the UK (range: 20.6% to 29.1%). The influence of gender, age and other potential confounding factors is being investigated and will be presented at the conference.

Conclusions: (1) Differences were observed in the prevalence of *S. aureus*, isolated from general practice patients, in nine European countries. Moreover, (2) within these countries large differences were observed between the participating general practices. (3) *S. aureus* susceptibility results from the Netherlands and several other participating countries will be discussed.

P066

The screening of *Brucella ceti* in harbour porpoises (*Phocoena phocoena*) stranded on the Dutch coast

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Introduction: *Brucella ceti* is detected in harbour porpoises (*Phocoena phocoena*) in countries around the North Sea, but not yet in animals along the Dutch coast.

Methods: Stranded harbour porpoises along the Dutch coast were submitted for necropsy at the Faculty of Veterinary Medicine (Utrecht University). Selected organs and lungworms were sent to the Central Veterinary Institute (CVI) for analysis for Brucella spp. In total 50 different animals from different locations along the Dutch coast were screened. In total 111 samples were analysed. All samples were cultured using methods described for isolation of Brucella species by the OIE (Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2010). The cultured samples were then screened by a PCR, based on the IS711 element. The positive isolates were typed using a published Brucella Multi Locus Variable Tandem Repeat Assay (MLVA, Le Fleché et al, 2010). Reference strains of *Brucella ceti* en *Brucella pinipedis* were included.

Results: In two of the 50 animals (4%) Brucella spp were found. In one animal Brucella was detected in the lungs and in the other animal in the bronchus. The Brucella species was typed as *B. ceti*.

Conclusions:

1. *Brucella ceti* is present in marine mammels stranded at the Dutch coast.
2. These findings extend the range as *B. ceti* was detected along the coast of England, Scotland and Belgium.
3. Zoonotic implications of the marine brucella spp are unclear and should be investigated further.

P067

Development of a rapid strategy to determine the toxin gene and virulence trait content in *Escherichia coli*

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Introduction: Many *Escherichia coli* strains can produce one or more toxins and can harbor other virulence traits e.g. shiga like toxins I and II (*stxI, stxII*), *E. coli* attaching and effacing gene (eae), heamolysin gene (*hlyA*) and the K1 capsular polysaccharide. Various clinical pictures, such as food poisoning, gastro-enteritis, urinary tract infections and meningitis, can be found when a patient is infected with an *E. coli* strain which produces one or more of these toxins/ virulence traits. Some infections may result in haemolytic-uraemic syndrome (HUS), which is a very serious complication. For surveillance purposes Dutch hospitals send clinically relevant *Escherichia coli* strains to the National Institute for Public Health and the Environment (RIVM) for the determination of toxin genes and virulence traits. First, the determination relied on 4 separate PCRs: 1) targets *stx I, stxII* variant a to g, hlyA and eae, 2) *StxII* variant f together with 16s i.e. an internal control, 3) the K1 capsular polysaccharide and 4) O157 and H7. Which PCR will be performed depends on the clinical picture. In the newly developed PCR described here only one multiplex PCR is performed targeting the same targets, the target which acts as an internal control is *adk*, a housekeeping-gene.

Methods: A subset of 205 isolates was selected. The selection was based on 175 toxin positive *E. coli* strains that were send to the RIVM during 2010-2011 and 30 strains

from a ring trial. All strains harbored one or more of the above mentioned targets. For molecular characterization of the strains and validation of the newly developed multiplex PCR both methods were performed on all strains. The 4 separate PCRs were visualized by capillary electrophoresis on the Qiaxcel-apparatus. The multiplex PCR, whose amplification products were generated with labeled primers, were separated by capillary electrophoresis on an automated sequencer.

Results and conclusion: Among the 205 strains, 183 strains were tested with the STEC PCRs. Sixty-three, (34%) were positive for eae, 48 (26%) isolates were positive for the heamolysin gene (hlyA), 44 (24%) were found to be stxI positive, 37 (20%)contained the gene for *stxII* variant a to g. *StxII* variant f and adk revealed a positive result for 20 (11%) and 178 (97%) isolates, respectively. Seventy-eight of the 205 strains were tested for confirmation of serotype O157:H7 with duplex PCR. This revealed a positive result for 18 (23%) O157 and 26 (33%) H7 strains, respectively. From the 51 strains for K1, 24(47%) were positive. These results were found with both PCR methods. For the targets used as internal spicies specific controls, i.e. 16s and *adk*, different results were found. This is due to the fact that these targets reveal strong resemblance in shigella spp. and other *Enterobacteriaceae*. We conclude that the new multiplex pcr has a high sensitivety and is specific for detecting the toxin genes and virulence traits of *E.coli*.

P068

Indentifying potential next steps in adaptation of *Bordetella pertussis* to vaccination by high-throughput sequencing

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Introduction: *Bordetella pertussis* causes pertussis or whooping cough in humans, a disease which has re-emerged worldwide despite vaccination. The persistence and re-emergence of *B. pertussis* has been attributed to several factors including waning immunity and pathogen adaptation. A single nucleotide polymorphism (SNP) in a gene encoding pertussis toxin subunit A (*ptxA*) and a change in number of repeats in pertactin (*prn*) were associated with shifts in the *B. pertussis* population, suggesting that strains with these new genotypes conferred increased fitness. Later, the emergence of strains with a novel pertussis toxin promoter (*ptxP3*) was associated with increased notifications. Thus, small mutations may have a large effect on strain fitness and result in selective sweeps. However, other mutations may have contributed to changes in strain fitness. To investigate this we used whole genome sequencing to identify additional adaptations.

Methods: Illumina sequencing technology was used to sequence 343 clinical isolates from 19 countries and 5 continents isolated between 1920 and 2010.

Results: Phylogenetic analyses revealed no geographical clustering which suggest that pertussis is disseminated very rapidly globally and multiple times. We identified six loci with a high number of SNPs including *ptxA* and *ptxP* confirming that the latter genes may play a role in vaccine escape. Fourteen new SNPs, including four SNPs in known virulence factors, were identified in the current circulating *ptxP3* strains. These SNPs may represent next steps in adaptation. Furthermore the genome size was found to be decreasing. In most deleted regions pseudogenes were overrepresented but from 2000 we increasingly found strains in Europe that had deleted *prn* and occasionally strains that had deleted the *ptx* operon.

Conclusions: Our findings show that adaptation occurred by successive accumulation of relatively small mutations and not acquisition of novel genes. This suggests that *B. pertussis* is well adapted to humans and only fine tuning is required to persist in face of intensive vaccination. This fine tuning occurred by antigenic variation, changes in gene regulation and deletions of genes. Adaptation was found in many known virulence associated genes but also in genes with unknown functions. Further analysis of these cryptic genes may reveal novel ways of pathogen adaptation to vaccination.

P069

Chlamydia psittaci in wild birds in the Netherlands

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Introduction: *Chlamydia psittaci* is the causal agent of the zoonosis psittacosis (or ornithosis). Birds, especially psittacine birds and pigeons, are the main reservoir of the bacterium for humans. In birds *C. psittaci* can cause disease with symptoms like conjunctivitis, inactivity and weight loss, but most of the time birds are symptomless carriers. Humans get infected by inhalation of dried, contaminated excreta from birds. Psittacosis in humans is characterised by flue like symptoms followed by severe, sometimes fatal pneumonia that might require antibiotic treatment and hospitalisation. Little is known about the occurrence of *C. psittaci* in wildlife, especially in wild birds. The goal of this study is to find evidence for *C. psittaci* infection in wild birds submitted to the CVI primarily because of suspicion of intoxication.

Methods: Over the years 2010 and 2011 in total 202 birds, representing 26 species, were sampled via a cloaca swab. The bird species could be grouped together into five groups: waterfowl (114 birds), pigeons (7 birds), gulls (21 birds), crows (12 birds), birds of prey (30 birds) and others bird species (18 birds). Birds were sent to CVI to investigate the cause of death. From the swab DNA was extracted and tested in a real time Polymerase Chain Reaction (PCR) targeting the OmpA gene.
Results: In 2010, 90 samples were tested from 14 bird species; in the sample of one mute swan DNA of *C. psittaci* could be detected. In 2011, 112 samples were tested from 19 bird species; DNA of *C. psittaci* could be detected in two mute swans, two herring gulls, one northern gannet and two common buzzards. Over the two years seven (3%) of the 202 tested birds were positive for C. psittaci DNA. None of the seven tested pigeons (two Eurasian collared doves, four feral pigeons and one common wood pigeon) and none of the twelve crow species (six carrion crows, four rooks and two jackdaws) were tested positive.
Conclusions:
1. DNA of *Chlamydia psittaci* could be detected in wild birds in the Netherlands.
2. The overall percentage of *C. psittaci* DNA positive birds in this pilot is low (3%).
3. Further research is needed to value these results and its implications for public health.

P070

Predictive value of strains causing asymptomatic bacteriuria in women with recurrent urinary tract infections receiving prophylaxis

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Background: A significant proportion of women develop a recurrence following an initial urinary tract infection (UTI). The predictive value of asymptomatic bacteriuria (ASB) in developing a recurrent UTI (rUTI) has not been established yet. Currently, it is not known whether information from an asymptomatic sample is useful in guiding antimicrobial therapy. In this study, we wanted to determine whether ASB is predictive for the development of a UTI and whether *Escherichia coli* ASB strains, isolated in the month preceding an *E. coli* UTI, are predictive for the subsequent UTI-causing strain in terms of antimicrobial susceptibility and pulsed-field gel-electrophoresis (PFGE) pattern.
Methods: Data originated from the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study (n = 445): two randomized controlled trials on prevention of rUTI in non-hospitalized pre- and postmenopausal women, recruited from January 2005 to August 2007. In the present study, the cumulative probability of being UTI-free during the 15 months' follow-up for women with and without ASB at baseline was studied using Kaplan-Meier estimates (KME) and compared using the logrank test. Subsequently, Cox-regression analyses were conducted including the following potential confounders: age, number of UTIs in the year preceding enrolment, sexual activity, presence of complicating host factors, menopausal status and received prophylaxis.
Antimicrobial susceptibility and PFGE pattern of 50 *E. coli* strains causing a UTI were compared with that of the ASB strain isolated one month previously. The *E. coli* susceptibility to the following agents was determined: amoxicillin, amoxicillin-clavulanic acid, trimethoprim, trimethoprim/sulfamethoxazole, norfloxacin, ciprofloxacin and nitrofurantoin. In comparing the antimicrobial susceptibilities, the relationship between ASB and UTI strains was expressed in positive and negative predictive values (PPV and NPV respectively). PPV was the proportion of patients who had an asymptomatic isolate resistant to an antibiotic in whom the subsequent symptomatic isolate was also resistant to this antibiotic. NPV was the proportion of patients in whom the asymptomatic isolate was susceptible to an antibiotic and the symptomatic isolate also.
Results: When comparing the probability of being UTI-free during 15 months of follow-up between women with and without ASB at baseline, no difference was found (KME: 43% vs. 45%, p> 0.05). After inclusion of the confounding factors, ASB at baseline was still not predictive (Hazard Ratio: 1.07, 95% Confidence Interval: 0.80 - 1.42). At least 90% of the patients had a susceptible *E. coli* UTI strain when a susceptible asymptomatic *E. coli* was isolated in the preceding month, applying to all antibiotics tested (all NPV> 90%). Also for resistant asymptomatic isolates, high predictive values (≥ 75%) were found with the exception of amoxicillin-clavulanic acid (34%). Asymptomatic and symptomatic isolates had similar PFGE patterns in 70% (35/50) of the patients.
Conclusions: (1) The presence of ASB is not predictive for the development of UTI in women with rUTI receiving prophylaxis. However, (2) the susceptibility pattern of *E. coli* strains isolated in the month prior to a symptomatic *E. coli* UTI can be used to make informed choices for empiric antibiotic treatment in women with rUTI.

P071

Genome-wide identification of ampicillin resistance determinants in *Enterococcus faecium*

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Enterococcus faecium has become a nosocomial pathogen of major importance, causing infections that are difficult to treat owing to its multi-drug resistance. In particular, resistance to the β-lactam antibiotic ampicillin has become ubiquitous among clinical isolates. Mutations in the low-affinity penicillin binding protein PBP5 have previously been shown to be important for ampicillin resistance in *E. faecium*, but the existence of additional resistance determinants has been suggested. Here, we constructed a high density transposon mutant library in *E. faecium*, and developed a transposon mutant tracking approach termed Microarray-based Transposon Mapping (M-TraM), leading to the identification of a compendium of *E. faecium* genes that contribute to ampicillin resistance. These genes are part of the core genome of *E. faecium*, indicating a high potential for *E. faecium* to evolve towards β-lactam resistance. To validate the M-TraM results we adapted a Cre-lox recombination system to construct targeted, markerless mutants in *E. faecium*. We confirmed the role of four genes in ampicillin resistance by the generation of targeted mutants and further characterized these mutants regarding their cell morphology and resistance to lysozyme. The results revealed that ddcP, a gene predicted to encode a D-alanyl D-alanine carboxypeptidase, was essential for high-level ampicillin resistance and is important for maintaining cell morphology during division. Furthermore, deletion of ddcP sensitized *E. faecium* to lysozyme and abolished membrane-associated D,D-carboxypeptidase activity. This study provides a new perspective on the genetic basis of ampicillin resistance in *E. faecium* and has led to the development of a broadly applicable platform for functional genomic-based studies in this organism.

P072

Contamination or colonization: methicillin-susceptible *Staphylococcus aureus* ST398 of animal origin persists in humans

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Introduction: In the last 10 years in Dutch people an increase in the number of MRSA is observed. This increase is primarily due to MRSA, with sequence type 398 (ST398) and is in particular observed in rural areas and concerns in most cases people who have been in contact with farm animals or livestock. Based on whole genome sequence analysis of animal associated ST398 MRSA's, these strains seem to be poorly equipped to colonize humans. Other data show that nosocomial transmission is less likely than non-ST398 MRSA strains. We hypothesized that it could very well be that ST398 MRSA is frequently contaminating the nose of especially farm workers who are intensively in contact with livestock. To test this hypothesis we performed an artificial human nose inoculation experiment to study the survival of an animal ST398 MSSA strain in combination with a human *S. aureus* strain.
Methods: From twenty-two healthy participants we have determined the *S. aureus* carrier state. All people were decontaminated with mupirocin twice daily and chlorhexidine-containing soap once daily for a period of five days. After five weeks we determined if the volunteers were free of *S. aureus*. In the end sixteen participants were inoculated with a human *S. aureus* strain 1036 (isolate from an intermittent carrier) and a calf *S. aureus* strain 5062 (ST398 spa-type t034). Therefore we have applied 10*7 colony forming units (CFU) per strain in each nostril. Over a period of twenty-one days seven nasal swabs were taken and quantitatively cultured for *S. aureus* to determine survival of both strains in the human nose.
Results: A rapid decrease in bacterial load was observed for both strains in the first days after inoculation. The human strain 1036 was eliminated faster (median 14 days; range 2 - 21 days) than the animal strain 5062 (median 21 days; range 7 - 21 days) but this difference was not significant ($p = 0.065$). Bacterial loads were significantly higher for the animal strain on day 7 and day 21 (resp. $p = 0.012$ and $p = 0.015$). Three trends in the elimination of *S. aureus* were observed. One group (n = 4/14) eliminated both strains within twenty-one days, while another group (n = 5/15) shows no difference in bacterial loads until the end of follow-up. The third group (n = 5/15) shows a difference in bacterial loads after twenty-one days.
Conclusion:
1. The strain 5062 (ST398 *spa*-type t034) is able to persist in the human nose for 21 days.
2. Bacterial load decreases rapidly in the first days after inoculation.
3. 29% (n = 4/14) shows elimination of both strains within 21 days.
4. 36% (n = 5/14) shows no difference in survival between both strains.
5. 36% (n = 5/14) shows a difference in survival between both strains.

P073

The role of hydrogen peroxide resistance in pneumococcal virulence

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Streptococcus pneumoniae is a gram-positive, catalase-negative bacterium and is classified as a facultative anaerobe. It is a member of the normal human

nasopharyngeal flora, that colonizes the mucosal surfaces of the host nasopharynx and upper airway. *S. pneumoniae* can spread from the upper respiratory tract to other organs, which leads to serious disease such as pneumonia, otitis media, meningitis, and bacteremia. The bacteria is exposed to aerobic conditions in the oxygen rich environment of the airway surface but is thought to be under anaerobic or microaerobic conditions during invasive disease. S.pneumoniae encounters external and internal oxidative stress during its lifecycle. Major exogenous sources of reactive oxygen species (ROS) for *S. pneumoniae* are the production of hydrogen peroxide (H_2O_2) by other lactic acid bacteria in the nasopharynx and oxidative burst of neutrophils and macrophages. Moreover, *S. pneumoniae* releases large amounts of H_2O_2 during aerobic growth, which is produced by pyruvate oxidase (SpxB).

S.pneumoniae lacks canonical proteins identified in other bacteria that protect against oxidative stress. How S.pneumoniae, an aerotolerant anaerobic bacterium that lacks catalase, protects itself against oxidative stress is still unclear. In this work we investigated the possible mechanisms that enable the bacteria to cope with hydrogen peroxide stress.

We identified an operon which mediates resistance to this stress and plays a role in the establishment of long term invasive disease. The contribution of various ROS species generated by the host in the killing of *S. pneumoniae* is under debate. Therefore, we investigated in depth the mechanism by which host cells kill the mutant and to further understand the role of hydrogen peroxide stress resistant in virulence. Additionally, we explored the role of oxidative stress survival in other aspects of the pneumococcal lifestyle.

P074

Modeling metabolic pathways of nasopharyngeal microbiota of young children based on 16S rRNA sequence data: an exploratory study

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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, antibiotic action or vaccination), these pathogens or opportunists might have the chance to emerge and cause symptomatic infections. In a recently published paper by our group, we investigated the bacterial composition of 96 NP samples of 18-month-old children using 454-pyrosequencing of the 16s rRNA gene V5-V6 hyper variable region (Bogaert *et. al.*, 2011). The microbiota profiles consisted predominantly of three bacterial species (> 70%); *Streptococcus pneumoniae, Moraxella catarrhalis*, and *Haemophilus influenzae*. Based on the microbiota composition, we found that samples could be classified into four "nasopharynx types"(NP types), either dominated by one of these three potential pathogens (> 50%) or represented a more mixed setting. Studying the underlying bacterial interactions responsible for these profiles will give us insight in which conditions are favorable for a balanced, and hence, healthy microbiota. In addition, bacteria utilize and produce a wide range of metabolic products (e.g., lactate, ethanol and hydrogen peroxide), enhancing or reducing growth of other microbes. As was previously described for gut microbiota, this nutrient cycle may be one of the drivers of nasopharyngeal microbiota composition.

Aim: Identify important metabolic pathways, which drive the composition of microbial communities of the nasopharynx.

Method: Using the online database KEGG (www.kegg.com), we reconstructed metabolic pathways of the most dominant genera and species present in the nasopharyngeal microbiota identified by Bogaert *et. al.*. We downloaded orthologs tables for bacterial pathways from KEGG, multiplied the presence of genes with the number of acquired reads per bacterial species per NP type, and normalized for the number of genomes present in the KEGG database for each species/genus (e.g., the genus *Streptococcus* has 50 genomes in contrast to only a single *Moraxella* genome). This resulted in a measure of relative frequency for particular genes within the NP types.

Results and conclusion: The modeled relative gene frequencies of the four NP types differed for several genes and metabolic pathways. This work was performed as an exploratory study to assess whether we can predict the gene content based on 16S rRNA metagenomic sequencing, since full genome metagenomic sequencing on low density microbial communities is difficult, laborious, and costly. In the near future we will attempt full metagenomic sequencing on a subset of the samples. In this way, we aim to find patterns of metabolic pathways driving the microbial communities at the upper respiratory tract. Ultimately, it could allow us the promote and steer microbiota into a healthy state by targeted intervention strategies.

P075

Characterisation of small intestinal Streptococcus and Veillonella populations

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Introduction and objectives: The small intestine is the first region where food comes into contact with intestinal bacteria. Therefore, its microbiota is expected to have an important influence on the digestion of food and host physiology. Recently, high-throughput 16S ribosomal RNA (rRNA) profiling of the human small intestinal microbiota revealed a bacterial community enriched in *Streptococcus* and *Veillonella* spp., both in effluent samples collected from ileostomy subjects as well as lumen samples from healthy individuals collected via an orally introduced catheter. Since Streptococcus and Veillonella are also abundant in the oral cavity, this study focuses on in-depth characterization of and comparison between these bacterial populations in both ecosystems in order to elucidate their functional properties and interactions as well as their dynamics in time.

Materials and methods: Ileostomy effluent was collected at two time points with an interval of one year, while oral samples were collected only at the latter time point. Samples were plated on Mitis Salivarius (MS) agar and Veillonella Selective Agar (VSA) for selective isolation of *Streptococcus* and *Veillonella* spp., respectively. Bacterial isolates were identified by 16S rRNA gene sequencing and classification. Sequences were grouped into phylotypes, while strain diversity was assessed by amplified fragment length polymorphism (AFLP) analysis and Rep-PCR.

Results and discussion: Of a total of 546 isolates, 392 were identified as Streptococcus (253), Enterococcus (66), Veillonella (63), Bacteroides (5), and Lactobacillus (5). For Streptococcus and Enterococcus 3 and 2 phylotypes were identified, respectively, whereas the remaining genera were represented by a single phylotype. REP-PCR and AFLP analysis distinguished 9 genomic lineages for Streptococcus and 7 for Enterococcus. Interestingly, the Veillonella and Lactobacillus phylotypes as well as 3 Streptococcus lineages could be cultivated from both ileostomy effluent and the oral cavity. Moreover, only 1 Streptococcus and 4 Enterococcus lineages were cultivated from ileostomy effluent at both time points.

Conclusion: Streptococcus, Veillonella, as well as Enterococcus isolates were successfully obtained. Phylogenetic analysis of the isolates revealed multiple phylotypes for Streptococcus and Enterococcus that could be further divided into multiple genomic lineages, which demonstrates the high diversity of the small intestinal microbiota beyond the species level. The same bacterial phylotypes and genomic lineages were obtained from ileostomy effluent and the oral cavity, suggesting that the oral microbiota may serve as inoculum to colonize the small intestine. Only 1 Streptococcus lineage was cultivated from ileostomy effluent at both time points, indicating that these small intestinal populations display relatively high population dynamics at the strain level

The genome sequences of representative isolates of each genomic lineage are currently determined to elucidate the functional properties of these isolates as well as their potential microbial interactions.

P076

Structure-function analysis of the *S. pneumoniae* virulence-associated protein SP1298

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Introduction: The Genomic Array Footprinting (GAF) technology was applied to various animal models of pneumococcal infection, i.e. colonization, bacteremia and meningitis, to identify *S. pneumoniae* genes essential during carriage and disease. Recently, two highly conserved genes, SP1298 and SP2205, were consistently identified in all infection models. Both genes are annotated as DHH subfamily 1 proteins belonging to the superfamily of phosphoesterases of which the function is still unknown. The aim of this study was to unravel the function of SP1298 by structural modeling and characterization of the knock-out mutants using electron microscopy and different proteomic approaches.

Methods: Scanning electron microscopy was used to study the morphology of the fj1298 mutant. Sub-cellular fractionation was used to determine the localization of SP1298 and for linear trap quadrupole mass spectrometry (LTQ-MS) analyses in order to determine differences in protein composition in the cell wall fractions of *S. pneumoniae* strain TIGR4 wt and fj1298 grown to mid-log. The model of SP1298 was built using the automatic modeling script in the WHAT IF & YASARA Twinset. Subsequent visualization and analysis was also performed using this program.

Results: Electron microscopy studies on the fj1298 mutant revealed elongated structures or 'fused cocci'. Furthermore, shorter chain lengths were observed as compared to the wild-type. These findings led to the LTQ-MS analyses on the cell wall fractions. Interestingly, strong differences in protein composition were found between TIGR4 wt and fj1298. Sub-cellular fractionation studies confirmed that SP1298 is a cytoplasmic protein. The model for SP1298 was based on the crystal structure of Sh1221 protein from *Staphylococcus haemolyticus* (43% identity) of which the function is as yet unknown.

The protein was found to form a dimeric structure by hydrophobic interactions and the active site pocket appeared to contain an Mn2+ ion.

Conclusions:

-Inactivation of SP1298 results in shorter chain lengths and an aberrant bacterial shape.

-Strong differences in protein composition were found between TIGR4 wt and fj1298.

-The predicted structure consist of dimer and contains an active site pocket with an Mn2+ ion.

-SP1298 is a cytoplasmic protein, no indication was found that SP1298 is directly membrane associated.

P077

Pyrazinoic acid decreases the proton motive force, respiratory ATP synthesis activity and cellular ATP levels

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Introduction: Pyrazinamide is an effective first-line anti-tuberculosis drug whose mechanism is poorly understood. Pyrazinamide constitutes a pro-drug that becomes metabolized into pyrazinoic acid.

Results: In this report we investigate pyrazinoic acid in a sub-cellular assay using membranes from *Myocbcaterium bovis* BCG. We confirm that pyrazinoic acid strongly decreases the proton motive force across the cytoplasmic membrane and demonstrate that the drug acts directly on the membrane [1]. Pyrazinoic acid also significantly reduced ATP synthesis rates in a pH-dependent manner, with significantly enhanced activity at acidic pH values.[1] These results indicate that pyrazinoic acid may exert impact on bacterial viability by both breaking down the proton motive force required for uptake of nutrients and by blocking ATP production. Pyrazinoic acid interfered with human mitochondrial ATP synthesis as well and displayed comparable affinities for human and mycobacterial ATP synthesis. This suggests that the direct action of pyrazinoic acid on energy metabolism is non-selective and that the different pH environment of mycobacteria as compared to human mitochondria may in part account for the clinical safety of pyrazinamide as front-line drug.

Conclusion: Our results contribute to the understanding of pyrazinamide, an essential drug for efficient tuberculosis treatment. The sub-cellular membrane assay used here to characterize pyrazinoic acid may be used in screening systems for new anti-mycobacterial drugs targeting bioenergetic pathways.

Reference:

1. Lu, et al. Antimicrob. Agents Chemother. 2011;55: 5354-7.

P078

Sensitized emission FRET to study protein-protein interactions in *Bacillus subtilis*

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The role of protein complex formation to understand how biochemical processes are organized at the molecular level is of growing interest. Proteins operate more often in complexes than independent of each other and there is growing evidence that these complexes are frequently transient.

In this study we will examine interacting proteins *in vivo* in the gram-positive bacterium *Bacillus subtilis* using time-lapse microscopy. We will use Förster Resonance Energy Transfer (FRET) to study these protein-protein interactions at the single cell level. With the use of the fluorescent protein toolbox there are many possibilities to give the proteins of interest a genetically encoded fluorescent label. FRET will be measured here via sensitized emission, where fluorescence emission of the acceptor will be measured after excitation of the donor.

The glycolysis and citric acid cycle metabolic proteins are expected to show temporal expression and complex formation1 and are the topic of our study. These proteins will be fused to a donor or acceptor fluorophore and their interaction will be followed.

P079

Immunogenic antigens of *Staphylococcus aureus* associated with bloodstream infection

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Introduction: So far, attempts to develop an efficient anti-Staphylococcal vaccine in humans as an alternative for increasingly failing antibiotic treatment were unsuccessful. There remains a need to find a broad immunogenic bacterial antigen yet the currently available reports exploring the immunogenicity of *S. aureus* antigens in humans are limited both in number and scope.

In this study we sequentially investigate the IgG and IgA antibody courses against 57 Staphylococcal antigens in 22 patients with a *S. aureus* bacteremia, compare IgG levels between patients and non-infected controls and describe microarray data obtained in an *in vitro* blood infection model. We discuss the potential of immunogenic antigens for a vaccine component.

Methods: IgG (and for patients IgA) antibody levels against 57 Staphylococcal antigens were sequentially measured in serum samples of 22 adult bacteremic patients and 24 non-infected controls with known nasal carrier status

using a bead-based flow cytometry technique (xMAP®, Luminex corporation). All used Staphylococcal antigens were recombinant proteins.
For 54 out of 57 investigated Staphylococcal antigens, the presence of corresponding genes in isolates from all 22 patients was determined by PCR.
RNA from two clinical isolates was isolated after 0, 30, 60 and 90 minutes incubation in heparinized human blood. RNA expression was analyzed using the BµG@s microarray with 3973 genes according to the manufacturer's protocol (Bacterial Microarray Group, St. George's Hospital Medical School, London) Data was analyzed using dedicated software.
The non-parametric Kruskal Wallis test and Mann-Whitney U test were used to compare antibody levels between patients and control groups.
Results: Twelve of the 54 genes investigated with PCR were found ubiquitously present in all 22 clinical isolates: alpha toxin, clumping factor A and B, EsxA, glucosaminidase, IsaA, IsdA, lipase, LytM, Nuc, SA0688 and PrsA.
Of the 57 tested antigens, SA0688 and PrsA were associated with the highest median fold increase from initial to peak antibody levels for both IgG and IgA in bacteremic patients (SA0688: 5.05 fold increase for IgG and 2.07 for IgA, PrsA: 2.92 fold increase for IgG and 2.72 for IgA). All other antigens showed median fold increases smaller than 2.0 for both IgG and IgA, with the exception of a 2.51 median fold increase in IgA levels associated with SasG.
When peak IgG levels of bacteremic patients were compared to IgG levels of non-infected carriers and non-carriers of *S. aureus*, antibody levels against SA0688 and leukocidin F and S were highly significantly elevated in patients versus both control groups ($p \leq 0.0005$).
Four out of 57 tested antigens were ubiquitously upregulated in the transciptomes of two isolates incubated in human blood for up to 90 minutes: SA0688, IsaA, EsxA and SCIN. However, none of the tested antigens was upregulated twofold or higher compared to exponential growth in BHI broth.
Conclusion: The putative ABC transporter SA0688 is ubiquitously present in the genome of clinical isolates, expressed in human blood *in vitro* and induces the most significant rise in IgG levels in bacteremic patients. Of the 57 tested antigens this antigen would therefore be a potent candidate for a vaccine component.

P080

Transmission dynamics of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a burn centre during a six-month period of patient monitoring by molecular typing.

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Introduction: Patients hospitalized with burn wounds are at increased risk of developing microbial colonization and infection. Infections may lead to prolonged hospitalization, an increased morbidity and mortality and disturbed skin graft acceptance. Knowledge of colonization dynamics is limited and standard bacteriological techniques are not designed to detect and monitor transmission of bacterial strains. Therefore, the use of appropriate molecular typing methods may provide more insight into these dynamics and can result in the design of targeted infection prevention programs.
Objective: To obtain more insight into the transmission dynamics of *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) in a burn centre.
Methods: From February till August 2011 all (n = 136) patients admitted at the Burn Centre, intake specimens were routinely taken from the nose, throat, and perineum on admission and from wounds and infection sites on admission and thereafter twice a week. In addition, 56 nurse practitioners and surgeons of the Burn Centre/ICU were screened for SA and PA (nasal) carriage.
A cluster was defined as a group of at least two patients carrying an identical SA or PA strain. During a six month period, all SA and PA isolates were typed by MLST and AFLP.
Results: Forty-eight out of 136 patients (35.3%) were positive for SA at any moment during hospitalization. We identified 27 multilocus sequence types and 38 AFLP types. Ten clusters of SA-positive patients were observed (range 2-8 patients). One third of patients were SA carriers at admission. Half of these carriers became infected by their own endogenous SA strain and in at least 67% of the patients, evidence for exogenous SA infection was observed. Three health care workers (HCWs) from the burn wound Dept. shared an SA-strain with one of the clusters.
PA was found in 11.6% of patients. In 50% of them, an identical PA was found in both an intake sample at admission and in a wound, indicating endogenous infections. None of the HCW were PA carrier. In 44% of patients carrying PA, evidence for exogenous infection of PA belonging to two large clusters was observed.
Conclusions: The results of this study show a high rate of endogenous and exogenous infections during a six month period in burn wound patients. The routine practice of molecular typing demonstrates the endogenous and exogenous spread in a burn centre and opens the possibility of more targeted infection prevention interventions.

P083

Evaluation of a monitoring system for nosocomial pathogens in a burn centre by three molecular typing methods

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Introduction: Burn wound patients are continuously at increased risk of developing infection by endogeneous and exogeneous pathogens. Early recognition of colonization and concomitantly typing of the colonizing strain may provide early infection prevention.
Objective: To determine a suitable monitoring program for infection prevention purposes of *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) strains in a burn centre using molecular typing methods.
Methods: During a six-months period (February-August 2011) all SA and PA strains from 136 patients admitted at the Burn Centre, were isolated from the nose, throat, and perineum on admission and from wounds and infection sites on admission and thereafter twice a week. All strains were typed using High Troughput MLST (HiMLST) using NGS, AFLP and Raman spectrometry (RS) (SA only). Clustering of patients was defined as a group of at least two patients carrying an identical SA or PA strain.
Results: Using HiMLST, 12 clusters of patients carrying SA with unique sequence types (ST) could be defined. These ST-clusters comprising 2 to 8 patients can be subdivided into one to five AFLP types per cluster. This results in an average of 2.6 AFLP types per ST-cluster. If AFLP is used to cluster patients, these clusters can be subdivided into one to three ST per cluster. The number of ST per AFLP cluster is, however, lower than the opposite: 1.5. This implies a significantly higher discriminatory power of AFLP in contrast to HiMLST ($p = 0.0185$). Comparable results were found using RS instead of AFLP, although its discriminatory power is even higher.
Only three ST-clusters of patients with PA were found in which only one comprised two AFLP types. Conversely, both AFLP-clusters contained only one ST.
At least one SA clone was recovered in three patients once, and as such not recognized as an outbreak. This clone was denoted as an epidemic clone during an outbreak one month after the first isolation, without recovery during that month.
Conclusions: A high diversity of SA infections with a notable number of possible transmissions based on both HiMLST and, to lower extend, on AFLP and RS respectively, was found. Since AFLP and RS clusters can contain more than one ST, use of them in a monitoring system can result in a lower positive-predicted value to trace an outbreak. Moreover, AFLP data are prone to day-to-day variation and RS require strict standardized culture methods. HiMLST data however, can be stored easily without loss of quality and can be compared to custom-made and international databases.
In our view, the combination of HiMLST for continuously monitoring transmission and AFLP for occasional outbreak management is a feasible and reliable system for infection prevention.

P084

Persistence and antiviral resistance of Varicella-Zoster virus in hematological patients

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Introduction: Varicella-Zoster virus (VZV) infections are a relevant cause of morbidity and mortality in hematological patients and especially in hematopoietic stem cell transplant (HSCT) recipients. The present study aimed to investigate the prevalence and clinical significance of persistence and antiviral resistance by systematically analyzing all episodes of VZV diagnosed in our laboratory in pediatric and adult hematological patients between 2007 and 2010.
Methods: Patient charts were reviewed to document patient and disease characteristics. VZV loads were determined in all available clinical samples from the day of diagnosis and thereafter. Persistent VZV infection was defined as a VZV infection that lasted at least seven days. Analysis of resistance was performed in all patients with a persistent VZV infection by sequence analysis of the viral thymidine kinase and DNA polymerase genes.
Results: In total 89 episodes occurred in 87 patients of whom 66 were recipients of an allogeneic HSCT. Follow up samples were available in 54 episodes. Persistent VZV was demonstrated in 32 of these episodes (59%). Complications occurred in 16 of the persistent episodes (50%) versus 2 of 22 non-persistent episodes (9%). Mutations were found in 22% of the patients with persistent VZV, including patients with treatment unresponsive dermatomal zoster that progressed to severe retinal or cerebral infection.
Conclusion: In hematological patients, VZV related complications occur frequently, especially in persistent infections. Antiviral resistance is a relevant factor in persistent infections and needs to be investigated in various affected body sites when a clinical suspicion of treatment failure arises.

P085

Evaluation of eight commercial Hepatitis E virus specific IgM and IgG ELISA assays

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Background and Aims: Hepatitis E virus (HEV) genotype 3 is recognized as an emerging pathogen in industrialized countries. The currently commercially available HEV specific enzyme linked immunosorbent assays (ELISAs) are primarily designed for the detection of antibodies against genotypes 1 (Birma) and 2 (Mexico) and may not sensitively detect HEV genotype 3 or 4. Therefore we evaluated the performances of eight commercially available HEV serum antibody IgM and IgG specific ELISAs for the detection of both genotype 1 and 3 HEV infections in a clinical setting.
Methods: We used a sensitivity panel consisting of 88 well-defined samples from patients with PCR-confirmed HEV infection on basis of time after infection, immune status and genotype. A specificity panel was constituted of 10 serum samples of acute infections of hepatitis A, B and C virus, human cytomegalovirus, Epstein-Barr virus, B19 virus and 22 serum samples of healthy blood donors. The analytical performance of all eight assays was assessed by dilution series of sera with known HEV genotype 1 or 3 antibodies and the WHO HEV antibody reference standard. Statistical analysis was performed using SPSS v17.
Results: Based on the analytical performance, six assays were selected for further clinical validation. Mikrogen new IgG assay was considerably more sensitive than its comparators for genotype 3. Clinical performance was assessed using receiver operator curve (ROC) analysis, which resulted in highest area under the curve (AUC) of 0.974, 0.979 and 0.994 for the Wantai, Mikrogen_new and DiaPro HEV IgM assays, respectively. Cohen's Kappa coefficient showed highest concordance (> 0.8) for Wantai and DiaPro for IgM, Wantai and Mikrogen_new for IgG. Highest specificity was found for the IgM Wantai and Mikrogen old assay (> 99%). Highest sensitivity was found for DiaPro IgM assay (81%).
Conclusions:
1. Our study shows that current commercial HEV ELISAs can be used to diagnose HEV genotype 3 infection in a clinical setting.
2. Considering the high concordance of the assays and the availability of HEV RNA assays, there's no longer a need to perform serologically conformational testing in diagnostic settings.

P087

Modifying of *Staphylococcus aureus* surface by sortase A specific substrate

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Introduction: The emerging multiple resistance of *Staphylococcus aureus* to antibiotic treatment is becoming a major public concern. Therefore, the need for new therapeutics is increasing. The majority of *S. aureus* virulence- and colonization-associated surface proteins contain a LPXTG motif that can be cleaved by a membrane-bound transpeptidase, sortase A (srtA), whereafter they can be incorporated into the cell wall via a covalent bond. Hereby, sortase A is playing a key role in virulence. Therefore, we want to exploit the enzymatic properties of sortase A for a novel strategy to fight *S. aureus* infections. The strategy involves development of decoy substrates containing the recognition motif for sortase A (LPETG), which will be incorporated by sortase A into the peptidoglycan layer of living bacteria. It is aimed that by this approach the virulence properties of *S. aureus* can be modulated.
Methods: *S. aureus* 8325-4 (wild type and an isogenic sortase mutant, WT and *fjSrt* respectively) was exposed to fluorescently labelled synthetic sortase-substrate K(FITC)LPETG-amide during growth in a Luria broth (LB) medium. A scrambled isomer (K(FITC)EGTLP-amide) served as a negative control. The incorporation of fluorescent substrate into *S. aureus* WT and *fjSrt* mutant was determined by FACScan and fluorescence microscopy in different bacterial growth phases. 8325-4 (WT and *fjSrt*), N 315, Newman and two clinical isolates were used to study the kinetics of sortase A dependent substrate incorporation in different bacterial phases (exponential, post-logaritmic, stationary and late stationary). The bacteria were grown in LB medium until different phases and subsequently incubated with sortase substrate in srtA buffer. The fluorescence was determined by FACScan.
Results: WT and *fjSrt* 8325-4 *S. aureus* incubation during 24 hours in LB medium upon addition of 1 mM K(FITC)-LPETG-amide substrate resulted in highly fluorescent WT bacteria, as detected by fluorescence microscope and FACScan. After treatment with 1% sodium dodecyl sulphate (SDS), fluorescence was still present, indicating that the non-native substrate was covalently incorporated into the bacterial cell wall. Moreover, the bacterial perimeter revealed the highest fluorescence levels. Neither in negative control, nor in bacteria incubated with the K(FITC) EGTLP-amide substrate detectable fluorescence was observed. Furthermore, the 8325-4 *fjSrt* strain exhibited no significant fluorescence with either substrate.

8325-4 WT strain incubated until exponential, post-logaritmic, stationary and late stationary phase upon initial K(FITC)-LPETG-amide and K(FITC) EGTLP-amide substrates addition in LB medium, resulted in a cumulative incorporation of only K(FITC)-LPETG-amide.
To study the effect of growth phase on the rate of incorporation, five WT strains (three laboratory strains and two clinical isolates) were cultured in LB medium until the exponential, post-logaritmic, stationary and late stationary phase. Subsequent co-incubation of normalized bacteria with K(FITC)-LPETG-amide substrate in srtA buffer led to the K(FITC)-LPETG-amide incorporation by all tested strains. However, the majority of the bacteria demonstrated the highest incorporation peak during the stationary phase.
Conclusions:
1. 8325-4 WT strain can incorporate an exogenous sortase A substrate in a srtA dependent and cumulative manner.
2. The incorporation level of the exogenous sortase A specific substrate is growth phase-dependent and peaks at the stationary phase.

P088

The EurSafety Health-net EMR project: Antimicrobial resistance of *Escherichia coli* isolates collected from nine urology services in the Euregion Meuse-Rhine in 2009-2011

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Objectives: This study assessed the regional differences in antibiotic resistance of *Escherichia coli* isolated from nine urology services in the Euregion Meuse-Rhine. Differences in resistance in the Euregion pose a risk to cross border patient mobility and safety. Therefore, a current overview of resistance is needed, which may enable adaptation of treatment protocols.
Methods: A total of 421 *E. coli* isolates were collected: 126, 119 and 176 from the Belgian, German and Dutch urology services, respectively. Susceptibility testing was performed with the micro broth dilution method with susceptibility breakpoints according to the European Committee on Antimicrobial Susceptibility Testing. Putative extended spectrum beta-lactamase (ESBL) producing isolates were tested with a combination disk diffusion test to confirm ESBL production and ESBL positive isolates were further identified with an ESBL array and a PCR and sequencing reaction to determine the type of ESBL.
Results: Resistance of *E. coli* to amoxicillin varied 48% to 61% ($p < 0.05$) whereas resistance to amoxicillin clavulanic acid ranged from 24% to 39% ($p < 0.05$). Resistance to ceftazidime and cefotaxime was highest in the Belgian isolates (10%) and 3% in the Netherlands ($p < 0.05$). No resistance was observed against the carbapenems. Resistance to the quinolones was overall high with percentages varying from 20% among the Dutch isolates to 40% among the Belgian ones ($p < 0.001$). Resistance to nitrofurantoin was less than 5% and resistance to the folate antagonists ranged from 20% among the Dutch isolates to almost 40% among the Belgian isolates ($p < 0.05$).
The prevalence of ESBL producing isolates varied from 1.7% among the Dutch isolates to 7.6% among the German isolates ($p < 0.05$). Most ESBL producers contained the CTX-M ESBL of group 1 ($n = 16$). A TEM ESBL was found in 2 isolates.
For the empiric treatment of complicated UTI, the prevalence of antibiotic resistance may not exceed 10%. Taken into account this cutoff value several antibiotic agents are no longer appropriate for empiric treatment. This includes amoxicillin-clavulanic acid, the fluoroquinolones and the folate antagonist in all three subregions and also some cephalosporines in the Belgian subregion.
Conclusions:
1. In the Euregion Meuse-Rhine, we observed regional differences in resistance for *E.coli*. For most antibiotics the highest resistance was demonstrated among the Belgian isolates.
2. The prevalence of ESBLs was highest in the German part.
3. Differences in prevalence of resistance might be due to difference in use of antibiotics.
4. Options for oral empiric treatment are limited.

P089

High Throughput MLST – Bringing molecular typing to the next level

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Introduction: MultiLocus Sequence Typing (MLST) is a widely used system for typing microorganisms by sequence analysis of seven housekeeping genes. MLST protocols are published for many species and online databases have proven to be a powerful resource in studying the (global) epidemiology. The main advantage of MLST compared to other typing techniques is the unambiguity and transferability of sequence data. The main disadvantage is the high costs to generate the sequence data.

Objective: Here we introduce the High Throughput MLST (HiMLST) method that employs Next Generation Sequencing (NGS), which delivers large quantities of high quality MLST data at low prices.
Methods: The HiMLST protocol consists of two steps. In the first step the seven MLST targets are amplified by PCR in multi-well plates. During this PCR the amplicons of each strain are provided with a unique DNA tag, the Multiplex Identifier (MID). In the second step all amplicons are pooled and sequenced in a single NGS run (GS Junior, Roche). After the sequencing run, the MLST profile of each individual strain can be easily generated using its unique MID.
Results: With the HiMLST we have generated 96 MLST profiles in a single run. The HiMLST was employed for Legionella pneumophila, Staphylococcus aureus, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* and will be applicable to many other species. Moreover, the use of MIDs allows the combined sequencing of different species in a single NGS run. Currently, the HiMLST reduces the cost of MLST by a factor 10 compared to traditional methods. It is expected that the costs can be reduced further by introducing low volume PCRs and automated processing of reagents and sequence data. In this way, the HiMLST capacity can be doubled while retaining the high quality of the sequences.
Conclusion: The introduction of HiMLST paves the way for a broad employment of the MLST as a high quality and cost effective method for typing microbial species.

P090

Long-term prevalence of IgG and IgA HPV-specific antibodies in serum and cervical secretion in HPV vaccinated adolescent girls

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Introduction: Human papilloma virus (HPV) causes cervical cancer. Oncogenic HPV types are common sexually transmitted viruses and can infect women soon after their sexual debut. In the Netherlands, the bivalent HPV vaccine (Cervarix, HPV16/18) was included in the national immunization program in 2010 for girls 12 years of age accompanied by a catch-up campaign for girls 13-16 years of age. In vaccine studies, vaccine efficacy against cervical intraepithelial neoplasia (CIN) 3+ associated with HPV16/18 have been found to be 100%. Vaccine efficacy against persistent infection and CIN2+ for cross-reactive HPV types 31, 33 and 45 amounted to 84%, 59% and 50%, respectively. To provide insight into post-vaccination immune responses against seven high-risk HPV types we evaluated specific IgG and IgA antibody responses in cervical secretion (CVS) and serum.
Methods: In a HPV vaccine monitoring study, pre-vaccination (n = 302), one year (n = 212) and two years (n = 141) post-vaccination CVS and serum samples from girls aged 14-16 years and vaccinated in a 2+1 schedule with the bivalent HPV vaccine were available. CVS was collected using a tampon and checked for the presence of blood. CVS containing> 25 erythrocytes/µl were excluded from analysis. Samples were tested for IgG and IgA antibodies against HPV16, 18 and cross-reactive HPV31, 33, 45, 52, and 58 using a virus-like-particle-based multiplex immunoassay. Significant ($p < 0.05$) differences of geometric mean concentrations (GMCs, expressed in LuminexUnits/ml (LU/ml)), pre and post-vaccination, were calculated using a Mann-Whitney test. Correlations ($p \leq 0.0001$) between antibody concentrations in CVS and serum were calculated using the Spearman correlation coefficient (r).
Results: One year after vaccination, IgG antibody concentrations in serum for HPV16 and 18 were significantly increased ($p < 0.0001$) up to a GMC of 3164 LU/ml (95% CI, 2716-3686 LU/ml) for HPV16 and 1612 LU/ml (95% CI, 1359-1913 LU/ml) for HPV18 compared with pre-vaccination levels. Comparable antibody levels were found two years after vaccination. In CVS IgG antibody levels against HPV16 and 18 were around 60 times lower as compared to serum. Post-vaccination IgG antibody levels against cross-reactive HPV types in serum and in CVS were highest for HPV31 and 45. IgA antibody concentrations for HPV16 and 18 were detectable in both serum and CVS, but in CVS these levels were around 160 times lower. In CVS IgA levels for cross-reactive HPV types were not detectable. We found a positive correlation between IgG antibody concentrations in serum and CVS one year after vaccination for HPV16 ($r = 0.5814$) and HPV18 ($r = 0.4953$). Not every girl had detectable HPV antibodies after vaccination in her CVS despite a high HPV-specific antibody level in her serum.
Conclusion: The persistence of HPV vaccine specific antibody levels in serum seems to be high 2 years after the 2+1 vaccination schedule in 13-16 year old girls. After vaccination, transudation and extravasation of HPV-specific antibodies from the systemic circulation to the cervical mucosa are important immune mechanisms resulting in high correlations between antibody levels in serum and CVS. Future studies in persistence/prevalence of these HPV-vaccine immune responses are necessary as antibody levels especially for cross-reactive HPV types might wane over time.

P091

Identification of the putative major autolysin in *Enterococcus faecium* involved on cell separation, eDNA release and biofilm formation

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Enterococcus faecium (Efm) 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 (BF) formation in this specie are pivotal for the development of new strategies to prevent BF formation. In several other species, extracelullar DNA (eDNA) is a matrix component and contributes to BF attachment and matrix 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 aimed to reveal the contribution of eDNA to BF formation and to identify the major autolysin in Efm E1162 implicated in these processes. The role of eDNA in BF formation was analyzed in a polystyrene assay and a semi-static BF model. Using bioinformatics (based on gene annotation), 6 putative E1162 autolysin genes were predicted, and single crossover mutants were constructed. The mutants were analyzed for their capacity to form BF, lyse in Triton X 0.02% in liquid media and for chaining morphology. BF formation of wildtype was decreased by 50% when DNAse was added both in the polystyrene and semi quantitative assays at t=0. Insertional disruption of the Efm gene locus tag: EfmE1162_2692 was the only one that showed a significant change in phenotype, resulted in a significant decrease in BF formation, non- susceptibility to Triton X, and significant chaining compared to wildtype. Also, residual BF in this mutant was not further decreased by DNase treatment. This autolysin is present in all sequenced *E. faecium* strains and has a glucosaminidase super family domain and six lysM domains, which is predicted to have peptidoglycan binding function. The domain sequences are 100% identical to AtlA of E. faecalis. In conclusion, eDNA contributes to biofilm formation and stability in Efm E1162 and is most likely released by autolysin locus tag: EfmE1162_2692. Based on all the phenotypes tested we hypothesized that this is the major autolysin in Efm and we suggest to rename this to AtlA.

P092

Borrelia realtime multiplex PCR for diagnosis of Lyme disease in the Netherlands

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The realtime PCR for detection of *Borrelia* species described by Gooskens et al (2006), that has been extensively clinically validated, theoretically misses B. lusitanea. Since this last strain has been reported to occur in the Netherlands by the RIVM and we wanted to detect all possibly relevant *Borrelia* species occurring in the Netherlands, we extended the PCR described by Gooskens et al with extra target genes by multiplexing.

Experimental set-up: Three cultures of *Borrelia* species prevalent in Europe, were used to test different primer-probe combinations directed against different *Borrelia* target genes in order to create a multiplex PCR with ideally three different target genes and a control gene. DNA from the three strains was isolated and diluted to the extent that it could only just be reproducibly detected by the optimized Gooskens single-plex OspA PCR. Primers and probes for different regions of OspA, FlaB and 5S-23S IGS, that were designed based on publically available sequences, were tested and optimized for detection of all three strains with sensitivities approximating the OspA single-plex PCR. Primers were checked using melting analysis.

Results: Both for IGS and FlaB PCRs could be designed showing similar Ct values compared to the OspA PCR for all three *Borrelia* strains tested. None of the PCRs tested gave rise to better Ct values compared to the OspA PCR. However, when SYBR green was used for detection Ct values of all PCRs improved significantly. However, this detection method gave rise to aspecific PCR products on feces DNA isolates, expected to be negative for *Borrelia* and, therefore, abandoned as first line clinical option. After optimization the PCRs could be multiplexed in combination with standard PHV primers and probes without significant change in Ct values. However, upon quadruplexing the FlaB signal became very faint, although in different combinations in triplex PCRs it was still acceptable. A panel of 10 *Borrelia* positive tick DNAs from the Netherlands was tested with the quadruplex PCR, resulting in positivity for both OspA and IGS of all positive samples in the panel, with comparable Ct values compared to the RIVM PCR values. The FlaB signal from this multiplex was not evaluated, since it still needs some improvement. A sample of B. lusitaneae DNA provided by Hein Sprong was detectable with one of the FlaB PCRs tested but not the OspA PCR, as expected.

Conclusion: It is possible to develop multiplex PCRs (at least a triplex) for detection of a broad variety of *Borrelia* species without loosing sensitivity compared to the singleplex OspA PCR described by Gooskens et al. detecting B. lusitaneae species found in the Netherlands next to B. burgdorferi, B. afzelii, B. garinii and B. valaisiana. After final optimization and collecting

sufficient, preferably well characterised, clinical material the multiplex will be clinically validated.

P093

Analysis of thermoregulated gene expression in *Enterococcus faecium* and functional characterization of a proline-rich surface protein

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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 is often difficult to treat due to the increasing resistance of this pathogen to several 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 the mid-exponential growth phase 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*.

Full length EfmE1162_0376 and separately its N- and C-terminal domains were overexpressed with N-terminal His6-tags in *E. coli* and purified. Polyclonal antibodies against the full-length protein were raised in rabbits. These antibodies were used to determine the relative levels of EfmE1162_0376 in exponential and stationary phase cultures of *E. faecium* E1162 by flow cytometry. This showed that EfmE1162_0376 is present at highest levels in exponentially growing cells at 37°C, while levels of surface exposed EfmE1162_0376 were lower in stationary phase cultures or cultures grown at 25°C. The localization of EfmE1162_0376 during exponential phase growth was assessed using confocal microscopy. EfmE1162_0376 was found to be distributed exclusively towards the poles of the bacteria. Polar localization has been described in other bacteria, for instance to concentrate the adhesion machinery facilitating host invasion.

The purified N-terminal domain of EfmE1162_0376 but not the C-terminal domain was able to bind fibrinogen but not the other extracellular-matrix components tested using ELISA. This indicates that the N-terminal domain is functionally involved in fibrinogen-binding while the function of the repeat-containing C-terminal domain remains to be elucidated. Recently, several reports have highlighted the interactions between bacteria and platelets. We are currently using flow cytometry to study a possible interaction between EfmE1162_0376 with platelets and determine, if present, whether such interaction is directly or indirectly mediated through fibrinogen.

In conclusion, we have shown that the EfmE1162_0376 encodes a surface protein of which the structural gene exhibits upregulated expression in response to mammalian temperature. The protein can bind to fibrinogen suggesting that it may be involved in the interactions of *E. faecium* with its mammalian host during infection.

P094

Unraveling the mechanisms of antimicrobial therapy-associated diarrhea: Susceptibility profiles of butyrate producing bacteria

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Introduction: Antimicrobial therapy-associated diarrhea is a common adverse effect in antimicrobial therapy. About 15-25% is attributed to *Clostridium difficile* toxins but the majority of cases remain unexplained. Here the role of butyrate is investigated since it is essential for the colonic mucosa, and especially for water homeostasis. *Faecalibacterium prausnitzii*-group bacteria and *Roseburia* spp are the dominant producers of colonic butyrate. We hypothesize that antimicrobial therapy-associated diarrhea may ensue due to a deficiency of colonic butyrate when these butyrate producing bacteria are susceptible to treatment with antimicrobial agents.

Methods: Strains of *F. prausnitzii, Roseburia inulinuvorans, Anaerostipes caccae* and *Eubacterium* cylindroides were used in susceptibility testing against 21 antimicrobial agents.

Results: Especially *F. prausnitzii* and R. inulinuvorans were found to be highly susceptible to most of the tested antimicrobials (90% and 86% respectively). Fecal butyrate concentrations are known to be correlated with the fecal numbers of bacteria from the *F. prausnitzii*-group and *Roseburia* spp.

Conclusion: We conclude that most antimicrobials have the potential to decrease the numbers of the dominant butyrate producing bacteria. As a result colonic concen-

trations of butyrate are expected to be decreased, which could lead to the development of antimicrobial therapy-associated diarrhea. Further prospective studies are needed to elucidate the role of this potentially important novel mechanism underlying antimicrobial therapy-associated diarrhea.

P095

Chronic HEV infection in orthotopic heart transplant recipients

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Introduction: Hepatitis E virus is an RNA-virus particularly causing large outbreaks of waterborne acute hepatitis in Asia, Africa and India. In the past years autochthonous sporadic cases have been described in the Netherlands. Moreover, chronic HEV-infection has been associated with the use of immune suppressive therapy.
Aim: We studied the prevalence and clinical presentation of HEV-infection in heart transplant (HTX) recipients.
Methods: Included were all living patients transplanted in the Rotterdam heart transplant program of which a serum or plasma sample was available in the serobank. Screening was performed by real time PCR, based on amplification of ORF3 genomic region. In all PCR positive patients IgG and IgM serology (Wantai, Singapore) was performed. A case was defined as a patient with a positive HEV RNA PCR result, chronic HEV infection as HEV RNA positive> 6 months. All cases were evaluated extensively including additional history and physical examination, additional lab tests for liver dysfunction, repeated HEV-virology both of plasma and faeces, HEV-PCR in blood and faeces of spouse (if present), HEV-genotyping by sequence analysis, ultrasonography and liver histology. In all cases the time frame of infection was determined by retrospective testing of routinely stored serum and plasma samples at the Dept. of Virology.
Results: In total 263 HTX patients were studied, immunosuppresion was prednisolone and Tacrolimus based, in some cases combined with mycophenolate mofetil and everolimus. Overall seven cases were found HEV PCR positive, of which six were defined as chronic HEV infection; estimated point prevalence was 3% (7/263). No positive cases were detected in spouses. Cases were predominantly older males (6/7), with a median age of 52 years (range 39-63), HEV infection occurred median 8 years (range 1-20) after HTX whereas infections clustered in recent years 2009 (n = 1), 2010 (n = 5), 2011 (1). Genotyping showed all infections to be genotype 3, phylogenetic analysis showed no direct relation between the isolated viruses, thereby excluding a common source in our patients. IgM antibodies at presentation were positive in only 2/7 (29%) of cases.
Median follow-up after case detection was 9 months (range 3-24 months). Liver enzymes at last follow up showed a wide range of activity: median AST 112 IU/l (range 39-457), median ALT 158 IU/l (range 21-353), and median gamma-gt 256 IU/l (range 196-336). Liver biopsies in patients with chronic HEV infection showed inflammatory activity compatible with chronic viral hepatitis, F1-F2 fibrosis, and grade 1-2 steatosis according to the Brunt classification.
Conclusion:
1. Autochthonous sporadic acute HEV infection with genotype 3 in heart transplant recipients occurred in 3% of the population studied. The consequences of this infection vary from mild transient viraemia to severe potentially progressive hepatitis with a marked steatosis in the liver biopsy.
2. As the majority of cases would not be detected by IgM-HEV serology, we advice RT-PCR as preferred method to diagnose HEV infection in immune compromised patients.

P096

MLVA as an alternative for phage typing of Salmonella enterica subsp. enterica serovar Enteritidis

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Introduction: Salmonella enterica subsp. enterica serovar Enteritidis (SEnt) is a pathogen frequently responsible for outbreaks of gastro-enteritis. To elucidate outbreaks, an epidemiological subdivision of this serovar is indispensable. Phage typing has been the traditional strain typing method used in the Netherlands for surveillance of SEnt. Disadvantages of the phge typing scheme are e.g. its decreasing discriminatory power, the subjective reading of phage reactions, the need of continuous maintenance and its labor intensiveness Multilocus Variable-Number Tandem-Repeats Analysis (MLVA) is a PCR method based on the amplification and fragment analysis of five repeat loci. Advantages of MLVA are: it is fast, easy to perform, less labour-intensive and it yields unambiguous typing data. Here we demonstrate that MLVA is an alternative to type Sent.
Methods: For this study we typed 426 SEnt isolates which were sent to our lab for serotyping from 2007 up to 2011. All strains were characterized by both phage- and MLVA typing. MLVA using 5 VNTR- loci was performed in a multiplex PCR's, the fluorescent labeled PCR products were sized on an automated DNA sequencer and the raw data was converted into allelic numbers i.e no. of repeats. MLVA data were stored in a connected Bionumerics database. Phage types were designated by pattern recognition and

recorded on worksheets. The separate reactions of patterns which did not conform to the phage scheme, i.e. atypical strains, were recorded manually. Simpson's diversity indices (DI) were calculated to determine the discriminatory power of both methods. An overall comparison was made between the two typing methods regarding type-ability, discriminatory power data processing and their concordance.

Results and conclusions: All isolates were type-able with MLVA whereas phage typing was unable to type 2% of the strains and yielded 4.2% atypical strains. The DI's of MLVA- and phage typing were 0,91 and 0,89 respectively. We found that doubtfully designated phage types could be sub-divided by MLVA and consequently provided a better understanding of these phage types. Surprisingly, the concordance between phage and MLVA typing was fairly good. MLVA was easy to perform, less time-consuming and its automatically generated unambiguous data were easy to handle and store.

MLVA turned out to have a discriminatory power that is similar to phage typing and yields clearly defined and reproducible data that can be used for clustering and source identification, it can also be stored and exchanged easily.

MLVA can sub-divide strains significantly and is a usable and feasible typing tool for SEnt, an alternative and consequently a step forward in typing Salmonella enterica subsp. enterica serovar Enteritidis.

P097

The wondrous tale of a plum pit

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Introduction: The antimicrobial resistance rate in the Netherlands is low but the incidence is increasing. An infection with both extended spectrum beta-lactamase (ESBL) producing bacteria and vancomycin resistant enterococcus (VRE) was never diagnosed before in our hospital.

Methods: We describe a patient whose blood cultures yielded a VRE and an ESBL producing *Klebsiella oxytoca*. Furthermore he developed abdominal abscesses with VRE and ESBL producing *K. oxytoca* after an ileus due to a plum pit.

Case report: A 66-year-old man presented at the emergency room with abdominal pain. A CT scan was performed and disclosed a corpus alienum in the terminal ileum with enlarged bowel segments. Laparotomy showed a near perforation of the ileum due to a plum pit. He recovered quickly and was discharged after 6 days with amoxicilline-clavulanic acid orally and a CRP of 422 mg/L.

Two days later the patient was readmitted with abdominal pain and shock-like symptoms. A laparotomy was performed and revealed a four quadrant peritonitis with a perforation of the terminal ileum.

Antibiotic treatment was started on admission to the intensive care unit (ICU) consisting of piperacalline/tazobactam and a single dose of gentamicine. The first blood culture, drawn on admission, yielded a VRE. On day 6 a CT scan of the abdomen showed multiple abscesses, which were drained. Blood cultures drawn on the same day yielded ESBL producing *K. oxytoca*. Unfortunately, even after four punctions, undrained abscesses were still present on the CT scan. The culture of the drained fluid drawn on day 6 yielded both a VRE and an ESBL producing *K. oxytoca*. The subsequently drained abscess fluids drawn on day 9 and 21 days yielded only the VRE. Meropenem was started on day 6 of admission to treat the ESBL producing *K. oxytoca*, and linozolid was started on day 9 to treat the VRE.

On day 19 the patient was discharged from the ICU to the surgical ward. Again he made a relatively quick recovery. His drains where removed and on the CT scan performed on day 35 only two small abscesses remained, which could not be drained. The VRE was daptomycin (MIC: 4 mg/l) resistant and quinupristin/dalfopristin (MIC: 0.75 mg/l) sensitive. Unfortunately, the latter could not be used in the treatment of this patient due to delivery problems. To complete the six weeks treatment of the abscesses linezolid had to be continued for an additional two weeks. Linezolid is registered in the Netherlands for a maximum treatment of four weeks because of its side effects. We explained our considerations to the patient and received informed consent. After six weeks of treatment with meropenem and linezolid he was discharged in good condition.

Anamnestic and upon examination we found no source for these resistant microorganisms.

Conclusion: This case represents the increasing antimicrobial resistance rate, even in the community, in the Netherlands and the subsequent treatment difficulties. Unfortunately, we could not define a source for the resistant microorganisms causing infection in our patient.

P098

Determining the appropriate age for a second immunization with meningococcal serogroup C conjugate vaccine; an intervention study among Dutch teenagers

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Introduction: In 2002 a Meningococcal serogroup C conjugated (MenCC) vaccination was implemented into the Dutch National Immunization Programme (NIP)

for all children aged 14 months. In addition, a catch-up campaign was conducted between June and November 2002 during which all children between 1 and 18 years were invited to receive a single MenCC vaccination. Overall vaccine coverage was 94% and afterwards MenC disease disappeared in the vaccinated cohorts and even decreased dramatically in the non-immunized cohorts. It is suggested that the great success of the MenCC vaccination is primarily based on the catch-up campaign inducing large scale herd immunity by reducing the nasopharyngeal carriage of MenC bacteria in the population.

In the past years it has become clear that MenC-polysaccharide (MenC-PS) specific antibody levels decline rapidly after primary vaccination in young children. In addition, protection induced by a primary MenCC vaccination appears to be age-dependant: cohorts vaccinated at older ages (up to adolescence) reveal greater and longer lasting protection than those routinely vaccinated in infancy. Of importance, next to an increased risk of invasive MenC disease in young children, there is an increased risk of invasive MenC disease during adolescence. This suggests that a second MenCC vaccination may be needed to maintain the successful contribution this vaccine has made to public (child) health in the Netherlands. In order to establish what age would be most appropriate for this second MenCC vaccination, we recently (October 2011) started the second immunization MenC study.

Methods: Three age-groups were recruited consisting of healthy 10 year olds (n = 91), 12 year olds (n = 91) and 15 year olds (n = 86) respectively. All participants received a primary MenCC vaccination at an earlier age, either during the mass catch-up campaign in 2002 (12- and 15 years olds) or at the age of 14 months (10-year olds). Participants were vaccinated with the Dutch registered MenCC vaccine at the beginning of the study. Blood and saliva samples were collected prior to (T0) and 1 month (T1) after vaccination and will be collected 1 year after vaccination (T2, October 2012). Serum and salivary MenC-PS specific IgG and IgA levels and serum IgG subclasses and avidity will be measured using a fluorescent-bead-based multiplex immunoassay (MIA). In addition, functional antibody levels will be measured using the Serum Bactericidal Antibody assay (SBA).

Results: Of the 4667 parents approached, 481 (10.3%) provided favorable responses and their children were assessed for eligibility. We enrolled 268 (5.7%) participants of which 264 (98.5%) completed both visits at T0 and T1. We expect to be able to show the first preliminary results regarding serum and salivary MenC-PS specific antibodies at T0 and T1 in April 2012.

Conclusion: Serum and salivary MenC-PS specific antibodies at (T0 and) T1 might provide a first indication what age would be most appropriate for a second MenCC vaccination in the Dutch NIP.

P099

Antimicrobial resistance in respiratory *Haemophilus influenzae* isolates: Results of a surveillance study from 2005 till 2010 in the Netherlands

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Introduction: Respiratory tract infections (RTI) are frequently caused by *Haemophilus influenzae* and responsible for 75% of the global antibiotic use in this area. Because of this, an increase in antibacterial resistance among respiratory microorganisms has been notified worldwide, including *H. influenzae*. Since most RTI are treated empirically, antibiotic resistance complicates treatment, with β-lactamase production by *H. influenzae* as a well known treatment failure. Therefore, data on national antibiotic resistance for *H. influenzae* is important to optimize antibiotic use.

Methods: *H. influenzae* isolates were obtained from respiratory specimens of patients from Pulmonology Services, collected in 14 different hospitals from 2005 to 2011 as part of the antimicrobial resistance surveillance performed by the Dutch Working Group on Antibiotic Policy (SWAB). The Minimal Inhibitory Concentrations (MIC) were determined according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Intermediate isolates were classified as resistant. β-lactamase production was detected using the nitrocefin test.

Results: In total, 1606 *H. influenzae* isolates were collected. Overall resistance to amoxicillin over the 6 year period was 16%, while this was 6% for co-amoxiclav. The other tested antibiotics showed overall resistance prevalence of 21% for co-trimoxazole, 30% for doxycycline and 98% for clarithromycine. Trend analysis revealed that the level of resistance was not significant different over time for all of the antibiotics tested.

The 16% amoxicillin resistant isolates (n = 263) were further divided in β-lactamase positive en negative isolates in agreement with co-amoxiclav susceptibility. Overall prevalence of β-lactamase positive strains was 11% among the total *H. Influenzae* isolates, implying this as the principal mechanism for amoxicillin resistance. Interestingly, trend analysis showed a tendency towards a decrease in the number of β-lactamase-producing strains although no statistical significance was reached (p = 0.341). Five% of the amoxicillin/co-amoxiclav resistant isolates were β-lactamase negative. Furthermore, trend analysis showed a stable trend over time (p = 0.835). Possible resistance mechanisms are mutation in the penicilline binding protein (PBP) or a β-lactamase which was not detected by the nitrocefin test.

Conclusion: Based on these data, co-amoxiclav should be the antibiotic of choice for empirical therapy because i) its lowest overall resistant value (6%) and ii) efficacy against most β-lactamase producing H. influenzae strains. Furthermore there's a (non-significant) tendency that the prevalence of β-lactamase producing H. Influenzae strains is declining. A point of concern by using co-amoxiclav is resistance by β-lactamase negative amoxicillin/co-amoxiclav resistant strains, although the prevalence of these isolates seems relatively stable and low (5%).

P100

Surveillance of Extended Spectrum Beta-Lactamase and AmpC-betalactamase producing isolates in Dutch poultry, pigs, turkeys and cattle from 2007-2011

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Introduction: Resistance to extended spectrum cephalosporins (ESC) in *Enterobacteriaceae* is mainly mediated by the production of enzymes hydrolysing the beta-lactam ring of these antibiotics. These enzymes are called Extended Spectrum Beta-Lactamases (ESBLs) or AmpC beta-lactamases (AmpCs). The genes encoding these enzymes are predominantly located on mobile genetic elements (plasmids) and are therefore easily horizontally transmitted. ESBL/AmpC-producing isolates are increasingly found in human clinical isolates, which is partly explained by people travelling to Southern Europe and Asia. It is suggested that the increase of these isolates in human clinical isolates can also be explained by transfer of these isolates via the food-chain. The objective of this study was to characterise ESBL/AmpC beta-lactamases producing isolates obtained from poultry, pigs, turkey, calves and dairy cows collected in the years 2007-2011 to better understand the epidemiology of these isolates in food-producing animals.
Methods: Turkey isolates (n = 100) were collected only in 2011. Isolates from broilers (n = 1311), calves (n = 795), dairy cows(n = 940) and pigs (n = 1275) were collected from 2007 to 2011, as part of the Dutch national monitoring program on antibiotic resistance in food-producing animals. Indole positive *E. coli*-like colonies were randomly isolated from a MacConkey agar plate. Minimum Inhibitory Concentrations (MICs) were determined for cefotaxime by broth micro dilution method using the Sensititre system. All isolates with reduced susceptible to cefotaxime according to the EFSA epidemiologically cut off value (MIC> 0.25 mg/L) were further analysed for the presence of ESBL/AmpC genes by micro array, PCR and sequencing.
Results: The highest prevalence of cefotaxime resistant *E. coli* was found in broilers varying from 18% in 2007 to 21% in 2010 (data of 2011 are not yet complete whilst writing this abstract). The lowest prevalence was found in dairy cows, varying from 0% in 2007 to 0.8% in 2010. Prevalence per year in pig, turkey and calf isolates was never higher than 5%.
In broilers the following ESBL/AmpC-genes were detected: $bla_{\text{CTX-M-1,-2,-9}}$, $bla_{\text{SHV-2,-12}}$, $bla_{\text{CMY-2}}$, and $bla_{\text{TEM-20,-52}}$. $bla_{\text{CTX-M-1}}$ was detected in all animal species, and was by far the most common ESBL-gene in broiler and pig isolates. In addition to $bla_{\text{CTX-M-1}}$, $bla_{\text{TEM-52c}}$ was detected in veal calves, $bla_{\text{CTX-M-2}}$ in dairy cow isolates and $bla_{\text{CMY-2}}$ and $bla_{\text{SHV-12}}$ in pig isolates. To date, no plasmid-mediated ESBL/AmpC genes were found in turkey isolates. In addition to the plasmid-mediated ESBL/AmpC genes, point-mutations in the promotor/attenuator region of the chromosomally located *ampC* gene of *E. coli* were identified. These may also lead to reduced cefotaxime susceptibility.
Conclusion:
1. Prevalence of ESBL/AmpC-producing *E. coli* over the years seems stable in all animal species with the highest prevalence found in *E. coli* from broilers, which corresponds to the high prevalence (> 80% animals positive) found in former studies done (using selective methods to detect ESBL/AmpC producers) at farm level.
2. The level of ESBL/AmpC-producing *E. coli* derived from pigs, veal calves, turkeys and dairy cows is still much lower than in broilers.
3. $bla_{\text{CTX-M-1}}$ is most predominant and found in isolates of all animal species.

P101

Development and validation of a single-tube Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) scheme for *Klebsiella pneumoniae*

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Introduction: Genotyping of *Klebsiella pneumoniae* requires a high-resolution genotyping method with a fixed scheme that allows (1) long-term retrospective and prospective assessment, (2) objective result readout and (3) library storage for database development and exchangable results. A multilocus sequence typing (MLST) assay that fulfills these conditions is available but this is an expensive and laborious method. The aim of this study was to develop a multiple-locus variable number tandem repeat (VNTR) analysis (MLVA) scheme which allows a higher sample throughput at lower costs and less hands-on-time than MLST, yet with a resolution that is at least as high.

Methods: Tandem Repeats Finder software was used to search for putative VNTRs. These were tested for variability by PCR and sequence analysis using 12 clinical *K. pneumoniae* isolates and 2 reference strains. Subsequently, eight suitable VNTRs were included into a single-tube fluorescently primed multiplex PCR that allowed automated fragment size analysis. The type allocation scheme was made using Bionumerics software and optimized using 124 additional clinical isolates. MLVA was validated against the gold standard MLST using a subset of 95 clinical isolates.
Results: All 138 isolates together yielded 84 MLVA types. Using subcultures from one of the clinical isolates, we found that the VNTRs in the assay were stable under laboratory conditions. For 9 patients in the study series additional samples were available (obtained within 1-27 days after the original sample). The typing results for these additional samples were identical, indicating *in vivo* stability of the VNTRs. Grouping of isolates in a minimum-spanning tree by MLVA was highly concordant with grouping by MLST.
Conclusion: We have developed an MLVA scheme for *K. pneumoniae* that has a typing resolution comparable to that of MLST but is much less time-consuming. Our results position this MLVA scheme as a robust, high-throughput and relatively low-cost tool for *K. pneumoniae* epidemiology.

P102

Acanthamoeba castellanii as a model system to monitor virulence and phagosomal escape of pathogenic mycobacteria

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The causative agent of tuberculosis, *Mycobacterium tuberculosis*, is an intracellular pathogen that infects macrophages. During infection this pathogen modulates the bactericidal mechanisms of its host cell, thereby creating a niche in which the bacterium can survive and replicate. Recently, it has been shown that in addition to the inhibition of phagosomal maturation, *M. tuberculosis* is able to translocate from this compartment into the cytosol of the host cell at later stages of infection. Similar characteristics were shown for the closely related species *Mycobacterium leprae* and the fish pathogen *Mycobacterium marinum*. Studies in human macrophages and the amoeba *Dictyostelium* have shown that this process is dependent on the mycobacterial ESX-1 protein secretion system, which is essential for mycobacterial pathogenicity.
Phagosomal translocation can be visualized by electron microscopy. However, as this technique is highly laborious and time-consuming, we sought for a fast method to screen for mutants that are unable to escape the phagosome. Using the amoeba *Acanthamoeba castellanii* as a model system, we were able to find clear differences in intracellular localization of *M. marinum* strains, visualized by fluorescent microscopy. Within infected *Acanthamoeba*, we observed that wildtype bacteria were dispersed in the cell whereas ESX-1-deficient bacteria were highly clustered, indicative of localization within a vacuole. In addition, we found that an *M. marinum* transposon mutant of *mmar_1402*, a gene encoding the most highly secreted PPE protein, displayed an altered intracellular localization. Electron microscopy confirmed the phagosomal localization of this mutant. By Western blot, we found that the *mmar_1402* mutant was defective for secretion of the ESX-1 substrate ESAT-6, suggesting that the inability of the transposon mutant to translocate to the cytosol was a result of defective ESX-1-mediated protein secretion.
Together, our results show that *Acanthamoeba castellanii* can be used as a model system to monitor cellular localization of *M. marinum*. Infection of *Acanthamoeba* potentially allows for fast screening for bacterial factors that are either directly involved in phagosomal translocation or affect ESX-1 mediated protein secretion.

P103

Accurate detection of VIM, OXA 48, NDM, KPC and IMP carbapenemase-producing micro-organisms within the first 24 hours after hospital admission

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Introduction: Rapid increase of carbapenemase-producing *Enterobacteriaceae*, *Pseudomonas aeruginosa* and other non-fermenters is a serious threat in clinical patients.
Early detection of these multi-resistant micro-organisms is essential for preventing their spread.
In this study we developed a screening method directly from patient samples for detection of the most prevalent carbapenemases (VIM, OXA48, NDM, KPC and IMP). With this screening method we used two different broths and subsequently a new commercially available rapid ligation-mediated Real Time PCR. This procedure made it possible to obtain results within 24 hours after hospitalisation.
Methods: We used two Tryptone Soya Broths (TSB) (ErasmusMC, Rotterdam, the Netherlands) to screen patient swabs (rectum and throat) for carbapenemase-producing micro-organisms.
The two broths, consisted of 0.25 µg/ml ertapenem and 2 µg/ml ceftazidim. Moreover, both broths contained 0.5 µg/ml of vancomycin for inhibition of gram-positive bacteria. The use of two broths appeared to be necessary, because preliminary research showed that VIM related carbapen-

emase lacked growth in ertapenem broth. For this reason a ceftazidim broth was used for the detection of VIM.
Both broths were tested for minimal incubation time (14 to 18 hours at 35±2°C), detection limit (CFU/ml before and after overnight incubation), background (influence commensal flora after incubation) and the minimal bacterial load needed for a positive ligation-mediated Real Time PCR (Checkpoints, Wageningen, the Netherlands).
Results: Overnight incubation at 35±2°C turned out to be essential, because an high input of the target is needed for this ligation depending PCR. Dilution series showed one single bacteria was enough for major growth in both broths after overnight incubation resulting in a positive ligation-mediated Real Time PCR.
Furthermore, this study illustrates that commensal flora and varying incubation time between 14 and 18 hours did not have influence on the final results.
Conclusion: This study showed that it is possible to screen patients for most prevalent carbapenemase-producing micro-organisms within 24 hours after hospitalization by using two different broths and a ligation-mediated Real Time PCR.

P104

A correlation between LOS class and capsule type in Guillain-Barré syndrome development

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Introduction: Lipo-oligosaccharide (LOS) sialylation is the main pathogenic factor in C. jejuni induced Guillain-Barré syndrome (GBS), a post-infectious auto-immune disease of the peripheral nerves. Based on gene content and organization, several different LOS loci classes have been identified but genes involved in LOS sialylation are only found in LOS class A, B and C. Although strains with LOS class B and C are frequently isolated from GBS-patient stools, so far, only LOS class A strains are significantly associated with GBS development.
There is controversy about the role of the polysaccharide capsule of C. jejuni in GBS induction. Classical capsule based serotyping according to the Penner scheme has identified GBS associated serotypes in mainly clonal GBS strain collections of different origin. The HS:19 and HS:41 serotypes are the predominant serotypes in preceding GBS in Japan and South Africa, respectively. Previous capsule serotyping of our non-clonal, predominantly Dutch strain collection however showed diversity and no particular association with a certain serotype. Recently, Poly et al. described a multiplex PCR that distinguishes individual capsular types based on gene content in the capsule locus. In this study, using an expanded GBS strain collection and the multiplex capsule PCR method, we assessed if there is a correlation between LOS class and capsule type.
Methods: A group of 39 well-characterized C. jejuni strains isolated from stools of GBS patient and 171 age and sex matched enteritis reference strain were used in this study. The GBS related strains predominantly originate from Dutch patients. Two strains from the Netherlands Antilles, two Belgian strains and one strain from Bonaire were included. The enteritis strains all were of Dutch origin. Standard PCR was performed to determine the LOS class. Specific primer sets were developed for classes A1, A2, B, C, D and E, based on the DNA sequence of unique genes or regions of the LOS locus classes involved. A multiplex PCR method comprising three primer mixes able to recognize 17 different capsule loci was used to determine the capsule type.
Results: LOS class typing confirmed and strengthened association of LOS class A with GBS. Furthermore, a significant association for LOS class A, B and C and GBS was found when compared to enteritis. Amongst LOS class A strains, there was no differences in subclass when GBS strains were compared to enteritis strains. LOS subclass A1, however, was associated with GM1/GD1a like LOS, two structures that are highly associated with GBS development.
Multiplex capsule PCR analysis resulted in identification of 5 main capsule types: 1, 2, 4, 19 and 23/36 that as a group were significantly associated with GBS related strains.
Conclusion: We conclude that there is a correlation between LOS class A, B or C, capsule type 1, 2, 4, 19 or 23/36 and GBS development.

P105

Relation between surgical site infections in vascular surgery and nasal carriage of *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* is the most important pathogen in the development of surgical site infections (SSI). Patients who carry *S. aureus* in the nose are at increased risk for the development of SSI. In cardiothoracic and orthopedic surgery it has been shown that the risk for SSI can be substantially reduced by preoperative eradication of *S. aureus* nasal carriage. We performed a prospective cohort study in vascular surgery to determine the relation between nasal carriage and surgical site infections, as this has not been done before.
Methods: All patients undergoing vascular surgery in the Amphia hospital in 2010 were included. Preoperatively, patients were screened for *S. aureus* nasal carriage using a PCR technique and the presence of SSI was recorded based on criteria of the CDC.

Results: 335 patients were operated and screening was performed in 224 (64%). Of those, 55 (24.5%) were positive, 159 (71.0%) were negative and 10 (4.5%) were invalid. There were 4 *S. aureus* SSI in the 55 carriers compared with 6 in 159 non-carriers (p = 0.284). A stratified analysis revealed a significant increased risk in nasal carriers undergoing aortic reconstruction surgery (3 *S. aureus* SSI in 20 procedures versus 1 in 65 procedures in non-carriers, p = 0.039).
Conclusion: Nasal carriers undergoing aortic reconstruction surgery are at increased risk for the development of *S. aureus* SSI. These patients will probably benefit from perioperative treatment to eradicate nasal carriage. In peripheral vascular procedures no increased risk was observed but final conclusions cannot be made since the numbers were small.

P106

Added value of multiplex Luminex® xTAG-GPP testing in the diagnosis of infectious gastroenteritis

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Introduction: Infectious gastro-enteritis (GE) can be caused by a wide range of pathogens. The Luminex® Gastrointestinal Pathogen Panel (xTAG®-GPP) detects the most common GE causing pathogens, including bacteria and their toxins, viruses and parasites. Validation of this assay was performed in a multicenter study using a preliminary version of the assay. Meanwhile a CE-IVD approved version of the assay was launched.
Methods: A total of 465 fecal samples, submitted in November-December 2011 to the Clinical Microbiology Laboratory for detection of bacteria, viruses and/or parasites causing gastroenteritis, were collected and analyzed using the CE-IVD xTAG® GPP assay. The results were compared with the routine diagnostic results that were obtained by culture (bacteria) or multiplex real-time PCR (viruses and parasites).
Results: Altogether, 465 fecal samples have been collected for the prospective application. Campylobacter, *Clostridium difficile* toxins, and Norovirus were most frequently detected. Because of the limited collection period, not all targets have been detected. xTAG®-GPP missed some positive samples of which the targets were not present in the assay (e.g. sapovirus and Dientamoeba fragilis). Additional positives were found by xTAG®-GPP since the assay detects a broad variety of pathogens whereas the diagnostic test requested by the clinician focused on certain pathogens and because of increased sensitivity of xTAG®-GPP as compared to bacterial culture.
Conclusion: xTAG®-GPP will not detect targets that are not present in the assay. Here, that was mainly Dientamoeba fragilis, a parasite of which the clinical relevance still is questionable.
On the other hand, in small children additional Clostrium difficile positive samples were detected by xTAG-GPP that may not be of clinical relevance as well. A disadvantage of xTAG-GPP compared to real-time PCR is the lack of quantitative results. The multiplex xTAG®-GPP assay enables a generic approach of GE testing resulting in additional positives for which an inadequate diagnostic test was requested.

P107

An ex vivo porcine nasal mucosa explants model to study MRSA colonization

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Introduction: *Staphylococcus aureus* is a common human and animal opportunistic pathogen. Nasal carriage of *S. aureus* (and of the methicillin-resistant variant MRSA) has been identified as a risk factor for various infections. Nevertheless, nasal colonization is poorly understood and good model systems are lacking. However, pigs are readily colonized by MRSA, particularly ST398. Therefore an ex vivo model of pig nasal colonization was developed. This model was adopted from a study of the interaction of porcine viruses with the respiratory tract [Glorieux *et al.* 2007, J Virol Methods 142:105-12].
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 prepared using a biopsy punch and cultured at an air-liquid interface. Light microscopy, scanning electron microscopy (SEM), and apoptosis staining were used to evaluate the effect of the ex vivo culture on the integrity and viability of the explants at 0, 24, 48 and 72 h of cultivation. Mucosa explants were inoculated with 3 different MRSA ST398 strains (S0462 - pig isolate, S0385-1 and S0385-2 human isolates derived from an endocarditis patient; $3*10^8$ colony forming units (CFU)/ml) and incubated for 2h. Next explants were washed 3 times with PBS and colonization was assessed within 180 min after adhesion. Colonization of the explants was investigated by determining the number of CFU at 0, 30, 60, 90 and 180 min. Bacteria were isolated by scraping the mucosa layer, after washing with PBS,

using PBS with 0.1% Triton-X-100 followed by plating on blood agar plates. Bacterial localization was determined by immuno-histochemistry using anti-*Staphylococcus aureus* protein A and SEM.

Results: The explants were cultivated up to 72 h without significant changes in morphology, cell-cell contacts and three-dimensional structure. All strains adhered to the mucosal layer of the explants with comparable numbers of CFU (± $8*10^6$, time 0). Bacterial numbers of two strains (S0462 and S0385-1) showed a decline to $5*10^6$ and $4*10^5$ CFU/cm^2 during the first 30 min, respectively. The CFU of isolate S0385-1 remained stable during the last 150 min of the experiment, whereas isolate S0462 showed growth to $4*10^7$ CFU/cm^2. Isolate S0385-2 gradually decreased in number of CFU during the experiment to $2*10^2$ CFU/cm^2. Bacteria adhered to the surface of or between epithelial cells of the mucosa explants. Morphological and viability staining did not show any significant changes in epithelial layer of the explants during the colonization experiments.

Conclusion: This ex vivo model represents a potential powerful tool to study bacterial colonization in pigs. Porcine nasal mucosa explants can be maintained in culture for at least 72 h without significant morphological changes and loss in viability. Using this model we were able to observe different patterns of MRSA ST398 colonization.

P108

Clonal emergence of methicillin-resistant *Staphylococcus pseudintermedius* among dogs in the Netherlands

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Introduction: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is an opportunistic pathogen causing infections in dogs and cats and occasionally in humans. It is associated with pyoderma, wound infections and otitis externa. Acquisition of the SCC*mec* element, which harbours the *mec*A-gene, makes this organism resistant to all β-lactam antibiotics. Additionally, most MRSPs are resistant to almost all other classes of antimicrobials, making MRSP infections a therapeutic challenge. The estimated prevalence of MRSP in the Dutch dog population is 2%. The first isolate of MRSP in the Netherlands was found in 2004.

The aim of this study was i) to determine the annual number of first-isolates of MRSP in samples submitted to the Veterinary Microbiological Diagnostic Centre (VMDC) 2004-2011, Faculty of Veterinary Medicine, Utrecht, the Netherlands and ii) to describe the molecular epidemiology of MRSP in the Netherlands.

Materials and methods: The laboratory database of the VMDC was retrospectively analyzed for the number of canine clinical samples from which *S. pseudintermedius* was isolated (which includes the MRSPs and the methicillin-susceptible *S. pseudintermedius*, MSSP). All reported isolates were routinely phenotypically and biochemically confirmed to be *S. pseudintermedius*. Suspected MRSPs were tested for the presence of the *mec*A-gene by Real Time PCR.

For all patients the first isolate of MRSP was genotyped by Multi Locus Sequence Typing (MLST) and *spa* typing. A subset of 61 isolates of MRSP was selected for additional typing by Pulsed Field Gel Electrophoresis (PFGE) and SCC*mec*-typing. For this, 25 clinics that submitted MRSP-positive canine samples to the VMDC in 2007 (n = 15), 2008 (n = 23) and 2009 (n = 23), were selected based on geographic diversity. From each clinic 1-6 isolates were selected, depending on the number of first isolates.

Results and conclusions: The number of first isolates of MRSP per year that the VMDC isolated from clinical samples was 1, 1, 9, 63, 56, 73 and 56 respectively from 2004-2010. The number of first isolates of MSSP remained stable (in average 932). The absence of MRSP before 2004 and the sudden increase starting in 2006 shows that MRSP is an emerging pathogen. This sudden increase has also been reported in other European countries. The reason is unknown.

For every patient the first isolate of MRSP was genotyped (n = 268). This resulted into 15 distinct Sequence Types (STs), of which ST71 was by far the most predominant (73%), 9 distinct *spa* types and 9 isolates that were not typeable with current protocols for spa typing. The subset of 61 isolates showed that 31 strains (51%) were of MLST-type ST71, PFGE-type J, *spa* type t02 and SCC*mec*-type II/III. This clone seemed to be the most successful after the emergence of MRSP in 2006. A collaborative project showed that this genotype is the most common in Europe. Remarkably, another clone is dominant in the USA. The predominance of this clone in the Netherlands hampers studies on the transmission of strains in the Dutch dog population.

In conclusion: MRSP started to emerge in 2006 with clone ST71-J-t02-II/III as the most prevalent genotype over the years.

P109

The development of middle ware solutions for the connection of molecular diagnostics devices

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Objectives: A standard routine molecular diagnostic laboratory has currently implemented several real time amplification equipment, automated isolation devices and amplification set-up pipetting robots. Mostly devices from different vendors are present and all have a short technical life span. All these individual devices must be linked using an open MiddleWare software program.

Methods: The automation of molecular diagnostic hardware can be linked to a Laboratory Information System (ISMED) using Microsoft Access and Visual Basic for Applications. This includes text file based interaction between a database and different devices, the automated analysis of internal and run controls, as well as the validation of individual diagnostic parameters. The software is implemented around our laboratory routine diagnostics set-up.

Results: PCR Job-files from our LIS enables the selection of both qualitative and quantitative assays. These are linked with internal controls, positive and negative controls for various isolation devices (EasyMag, MP32, MP96). Simultaneously, information for CAS1200/Qiagility pipetting robots is generated, which can be used for the PCR set-up on ABI7500 amplification machines. Primer and probe batches and control reagents (internal and positive controls) with specifications (95% confidence intervals) are used for the validation of the results. Out of range data are located separately, while the accepted values are interpreted electronically with visual green/red colors results. For quantitative laboratory developed assays, values are automatically assigned based on validated and pre-calculated standard curves. Finally, using a PCR-RES file, the data are uploaded into our LIS. QC data are available in the database to enable time independent evaluation of reagents and controls

Conclusion: The implementation of an open software solution to connect equipment from different vendors has enabled us to reduce turnaround time, reduce mistakes, limits the use of copying results by using pen or pencil, enables the automated analysis and return of results, enabled the QC of laboratory developed tests (primer and probe batches, internal and positive controls).

P110

Seroprevalence of cytomegalovirus in the Netherlands and risk factors for infection

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Introduction: Cytomegalovirus (CMV) is an endemic virus capable of establishing lifelong latency. In healthy persons CMV infection does not lead to clinical symptoms, whereas in immunocompromised individuals CMV infection can cause significant morbidity.

CMV infection during pregnancy can lead to congenital CMV infection in the unborn child and is the most common congenital infection. About 12% of the congenital CMV infections lead to symptoms at birth (including cerebral calcifications and chorioretinitis) and in approximately 20% to long term sequelae, especially sensorineural hearing loss. The risk of congenital CMV infection is related to maternal serological CMV-status and the seroprevalence in the population.

The goal of this study was to determine the seroprevalence and risk factors of CMV infection in the Netherlands. In turn these data can be used as input for modelling scenarios to explore implications for primary and secondary preventive measures (such as future vaccination programs).

Methods: We assessed the seroprevalence of CMV in the Netherlands using a population-based serum bank (PIENTER-2) collected in 2006-2007. A total of 4774 sera from individuals (0-79 year) who participated in PIENTER-2, with an over sampling of immigrants (n = 646) were tested for CMV-specific IgG antibodies using ELISA (ETI-CYTOK-G PLUS DiaSorin, Saluggia, Italy). CMV seroprevalence rates for the overall population and several subgroups (age, ethnicity, and socio-economic status) were assessed using SAS.

Results: The overall seroprevalence of CMV in the general population (0-79 year) was 49%, increasing with age from around 30% in infants (< 1 year) to 70% in elderly (> 65 years).

There was a marked difference between the overall seroprevalence of the native Dutch population (42%) and non-Western immigrants (77%). Education and income were associated with seroprevalence, with a higher seroprevalence in groups with low income (63%) and low education levels (75%) compared to groups with high income (40%) and high education levels (39%). The seroprevalence of women of childbearing age (19 to 44 years) varied from 38% in women of Dutch origin to 88% in non-Western immigrants. In Dutch women of childbearing age the seroprevalence increased from 30% to 50% between the ages of 19 and 44 years. In addition women with children have higher seroprevalence rates (46%) compared to women without children (39%).

Conclusions:

1. Almost half of the population show serological evidence of prior CMV infection.
2. In accordance with the literature CMV infection in the Netherlands is more prevalent among non-Western immigrants and those with a lower socio-economic status.

3. The increase of seroprevalence in women of childbearing age shows that primary infections occur frequently during this period with a potential risk of vertical transmission and associated long term sequelae for the unborn child, in particular hearing loss.
4. These data can be used in modelling scenarios to evaluate the implications for primary and secondary preventive measures, such as future vaccination programs.

P111

Effect of storage conditions on the detection of HIV-1 minority viral populations in DBS using population-based genotyping

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Introduction: Using dried blood spot (DBS) sampling for HIV-1 drug resistance testing is convenient for surveillance and monitoring of HIV-1 drug resistance in resource limited settings (RLS). However, storage temperature and duration can lead to degradation of viral RNA in DBS samples, which could lead to reduced detection of less prevalent viral populations. We investigated the effect of storage temperature and duration on the detection of majority and minority viral populations at three different viral loads (VL).
Methods: Well characterized HIV-1 subtype B and C reference viruses were prepared to represent 1.00E+03 (low VL), 1.00E+04 (medium VL) and 1.00E+05 (high VL) RNA copies/ml. Whole blood (WB) was spiked with different proportions of these HIV-1 subtype B to C dilutions to represent 100:0, 90:10, 75:25, 50:50, 25:75, 10:90 and 0:100 percent. Spiked WB was spotted on 903 filter cards and dried for 3 hours. Cards were placed in zip-lock bags with desiccant and stored for different time periods (1 day [baseline], 1, 2 and 4 weeks) at +30°C and -20°C. At each time point, samples were extracted in duplicate and genotyped. Sequences were aligned and at each of the subtype discriminatory positions, genotype was assigned as pure subtype B, mix of both subtypes, or pure subtype C. Percentage of genotype was determined.
Results: Over the four time periods examined, amplification was more successful for samples stored at -20°C compared to +30°C. High VL samples showed minimal effect of storage temperature on majority and minority viral population detection, with similar results observed between baseline and week 4. Detection of both subtypes in medium VL samples decreased over time, predominantly in samples stored at +30°C. Increasing storage duration increased the prevalence of pure subtypes at +30°C. The overall sequencing success rate for low VL samples also decrease with longer storage duration.
Conclusions: At high VL, detection of majority and minority viral populations within a sample is stable at both -20°C and +30°C, at least up to 4 weeks. Storage at +30°C has a negative impact on amplification of HIV-1 from DBS, especially at lower VL. Over time extreme conditions (+30°C) lead to degradation of viral RNA, resulting in a greater stochastic effect on amplification and subsequent selection of a single species. This suggests that when the number of viral copies decreases, there is selective amplification and loss of minority viral population detection.

P112

The nasopharyngeal microbiota in relation to 7-valent pneumococcal conjugate vaccination: a randomized controlled trial in healthy children in the Netherlands

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Background and aims: 7-valent pneumococcal conjugate vaccination (PCV-7) is implemented in the National Immunization Program in the Netherlands and effective against vaccine-serotype invasive disease and colonization. However, replacement with non-vaccine pneumococcal serotypes and other potential pathogenic residents of the nasopharynx occurs. In view of (future) vaccine-effectiveness and -development it is important to study the breadth of those shifts in more detail.
Methods: We characterized nasopharyngeal microbiota profiles of 100 vaccinated (PCV-7 at 2, 4 and 11 months) and 100 unvaccinated children participating in a randomised controlled trial in the Netherlands performed in the pre-PCV7 era. Children were sampled at 12 and 24 months of age. Bacterial load was determined by universal Real-Time PCR and microbiota composition by GS-FLX-Titanium-Sequencing of 16S-rRNA gene amplicons spanning the V5-V7 regions.
Results: Overall, we found 13 phyla and 280 Operational taxonomic Units (OTU), with Moraxella, Haemophilus, Streptococcus, Dolosigranulum and Corynebacterium as predominant genera. PCV-7 vaccination was associated with an increase in bacterial load and OTU diversity, showing a significant increase in Veillonella, Prevotella, Actinomyces and other streptococcal OTUs at 12 but not at 24 months of age. Though not significant, we

also observed a trend towards increased presence and abundance of Haemophilus and Staphylococcus in PCV-7 vaccinated children at 12 months of age.
Conclusions: We observed a temporary effect of PCV-7 on nasopharyngeal microbiota composition in 12-month-old children 1 month after the last dose. Duration and implications of those effects for example regarding respiratory health deserve further studies.

P113

The MagNA Pure96-LightCycler 480 384-well system for detection of gastroenteritis in faeces samples

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The demand for molecular diagnostics on samples from patients with symptoms of gastroenteritis is substantial and still increasing. In addition, the extensive number of different pathogens to be analysed, makes optimization of sample processing essential. Furthermore, sample tracking and decrease of hands-on time requires process automation. Aim of this study was to compare DNA extraction/detection efficiency using two different lines: The nucleic-acid purification system MDx-BioRobot (Qiagen) combined with AB7500 (96-well real-time PCR; Applied Biosystems), and the purification system MagNA Pure96 (Roche) combined with LightCycler 480 (384-well format; Roche).
Routine, clinical stool samples were used to screen for the following pathogens: *Salmonella* spp., *Shigella* spp., *C. jejuni*, *C. coli*, Shiga-toxin producing *Escherichia coli* (STEC), *C. difficile*, *G. lamblia*, *C. parvum*, *E. histolytica* or *D. fragilis*. Samples were pre-treated by robustly mixing in a buffer and supernatants were extracted using the extraction robot specific protocols. Eluates and reaction mix were transferred into PCR-reaction plates by using a CAS1200 (Corbett Life Science) and analysed by multiplex PCR.
First, serial dilutions of target DNAs and feaces samples containing different pathogens demonstrated an equal analytical sensitivity for both detection systems. To further asses the sensitivity and specificity we prospectively tested 724 stool samples. Of the positive samples 99.6% matched in both lines. The evenly divided discrepancies were mainly seen in samples carrying low target loads. Analyses on the internal extraction control (PhHV) demonstrates a decrease in inhibition (5.4% vs. 3.9%), and a tremendous reduction in competition (23.4% vs. 0.2%) in pathogen-positive samples, when the MagNA Pure96-LightCycler 480 was used.
In conclusion, the 384-wells PCR-system in combination with the MagNA Pure96 maintains analytical and clinical sensitivity and specificity whereas competition and inhibition of multiplex PCR reactions have substantially decreased. This enables further automation of molecular diagnostics of gastroenteritis in clinical faeces samples.

P114

Two patients with pulmonary embolism associated with Mycoplasma pneumoniae infection

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Introduction: *Mycoplasma pneumoniae* can cause a wide variety of extrapulmonary diseases, thrombosis however is very rare. In December 2011 we diagnosed two cases of pulmonary embolism in patients with *M. pneumoniae* pneumonia.
Case 1: A 33-year-old woman, who was 23 weeks pregnant, presented at the emergency Dept. with a cough for three weeks, dyspnea and chest pain. She had no fever. She was treated by her general practitioner with amoxicillin without effect. On admission her C-reactive protein (CRP) was 18 mg/L, the white blood cell count (WBC) was elevated 12 /nl, platelet count was normal. A chest X-ray revealed bilateral pulmonary infiltrates. Computed Tomography Pulmonary Angiography (CT-PA) demonstrated pulmonary embolism and bilateral pulmonary infiltrates. She was treated with fraxiparin and erythromycin. Polymerase Chain Reaction (PCR) on bronchial lavage fluid was positive for *M. pneumoniae*. After two weeks of hospitalization she was discharged in good condition. Until this moment no adverse effect on the pregnancy is noted.
Case 2: A 27-year-old man presented at the emergency Dept. with fever since two weeks and worsening dyspnea since one week. He was treated by his general practitioner with cotrimoxazole and doxycylin. On admission his CRP was 51 mg/L, WBC was 12.4 /nl, with increased atypical lymphocytes in the peripheral blood smear, the platelet count was 441 /nl. The chest X-ray demonstrated bilateral pulmonary infiltrates. He was admitted and treatment was started with cefotaxim and ciproxin. After four days his condition worsened and he was transferred to the Intensive Care Unit for mechanical ventilation. An increased D-dimer level of 24.69 µg/mL was detected. The CT-PA demonstrated bilateral massive central pulmonary embolisms. Anticoagulation treatment with fraxiparin and antithrombolytic treatment with urokinase was started. Both PCR on bronchial lavage fluid and serology (IgM and IgA and complement fixation titer 1:40) were positive for *M. pneumoniae*. The cold agglutinin titer was high 1:512. Antimicrobial treatment was then switched to doxycycline.

Further analysis of risk factors for thrombosis revealed hyperhomocysteinemia. After 18 days of hospitalization he was discharged in good condition.

Conclusion: We present two patients with pneumonia due to *M. pneumoniae* and pulmonary embolism. Both patients recovered after starting anticoagulation and antimicrobial treatment. Both patients had other risk-factors for pulmonary embolism; pregnancy in the first case and hyperhomocysteinemia in the second case. The mechanism underlying thrombosis due to *M. pneumoniae* remains unknown, but most likely multiple pathomechanisms are involved.

P115

Lactate dehydrogenase, a novel drug target in *Schistosoma mansoni*?

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With a global infection rate of over 200 million, an at-risk population of 700 million, Schistosoma infections pose a significant burden on global health. Treatment of schistosomiasis currently relies on praziquantel, but unfortunately this drug seems to lose its efficacy as emerging resistance is reported by recent field and laboratory studies. Vaccination is being researched, but a working vaccine is still far away.

However, the unique metabolism of *S. mansoni* could allow for a new approach to treating this severe parasitic infection. During its lifecycle, *S. mansoni* makes transitions from free living to parasitic stages. Upon penetration of the mammalian host, the metabolism switches instantaneously from the aerobic metabolism of the free living cercariae to the predominantly anaerobic one of the adult parasite. Free-living stages of *S. mansoni* use as energy source their endogenous glycogen stores, which are aerobically degraded via the Krebs cycle to carbon dioxide. When cercariae penetrate the final host and transform into schistosomula, they switch immediately from carbon dioxide production via the Krebs cycle to lactate production via glycolysis. Lactate remains the main end product of glucose degradation during further development into the adult stage.

A key enzyme in this instantaneous metabolic switch and in the continuing homolactic fermentation of the adult parasite is lactate dehydrogenase (LDH). Schistosomal LDH (*SmLDH*) differs from other eukaryotic LDHs, including those of the final host. *SmLDH* is inhibited by physiological concentrations of ATP and this inhibition can then be abolished by intermediates of glycolysis. In this respect *SmLDH* bares more resemblance to many prokaryotic LDHs than to eukaryotic ones. The kinetic difference with the LDH of the host, which is mirrored in differences in primary structure of *SmLDH*, makes LDH a potentially very interesting drug target, as anaerobic glycolysis is for this parasite the only pathway available to generate ATP.

Therefore, we further investigated this rather unique eukaryotic LDH. RNA was isolated from *S. mansoni* and cDNA was transcribed. Using nested primers SmLDH wild type (*SmLDH-WT*) was cloned and expressed in *E. coli*. The sequence of *SmLDH-WT* was mutated to *SmLDH-2M* by specifically mutating two sites, V185C and Y188W, changing a valine to a cysteine and tyrosine to tryptophan respectively. Both enzymes were subsequently purified by nickel-affinity purification using an incorporated 6xhistidine site. Enzyme activity was tested by measuring the conversion rate of NADH to NAD+ under influence of ATP. 1. Our results showed that the activity of *SmLDH-WT* is increased 4-fold in the presence of activator. 2. Furthermore, 5mM ATP completely inhibited enzyme activity.

3. When the enzyme was inhibited by 5mM ATP, activation resulted in a restoration of 33% of the normal enzyme activity. 4. We showed that the mutant *SmLDH-2M* could no longer be reactivated.

5. These results clearly indicate the importance of valine 185 and tyrosine 188 for the reactivation of ATP-inhibited SmLDH. 6. This unique binding site could be an important target in treating schistosomiasis.

P116

Public health implications of introduction of PCR. Lack of uniformity in reporting Shigatoxin-producing *Escherichia coli* and Shigella to and by the municipal health service

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Introduction: Since the introduction in 2007 of molecular diagnostics for Shigatoxin-producing *Escherichia coli* (STEC) and later on for *Shigella*/entero-invasive *Escherichia coli* (EIEC), the number of notifications to municipal health services (MHS) has risen dramatically. Detection of Shigatoxin-genes is a more sensitive but less specific detection technique than culture of STEC. However, microbiological and clinical implications are still unclear and therefore health authorities are unnecessarily burdened. The surveillance for this pathogen is severely hampered. In June 2011 an algorithm has been proposed including the use of, polymerase chain reaction (PCR) cycle threshold (Ct) value of ≤ 35, as a diagnostic tool for STEC to guide control measures taken by MHS. To investigate the problem and the implementation of the proposed algorithm, a questionnaire has been held under all MHS of the Netherlands.

Methods: In December 2011 a telephone questionnaire has been held under 28 MHS. Listed was: 1) how many MHS are dealing with PCR results of STEC and *Shigella*, 2) how many molecular results of STEC and *Shigella* were notified by medical microbiology laboratories (MML) to the MHS during the first of January until the first of November 2011, 3) which criteria are used by MHS to report to the national health authority and 4) which arrangements have been made with local MML.

Results: Of the 28 MHS 23 are dealing with STEC PCR results. Five MHS only deal with culture results of STEC. The number of notifications varies from 1 to 118 between the different MHS over this time period. Criteria to report vary from only reporting culture proven molecular STEC results to reporting all positive PCR STEC results. Two MHS also have redefined their case-definition in different ways. Only 6 of all 28 MHS report and take control measures as defined by the previously proposed algorithm. Twenty MHS are dealing with *Shigella*/EIEC PCR results, whereas 8 only deal with culture results. The number of notifications varies from 3 to 73. Fifteen MHS only report culture proven Shigella, whereas 13 also report positive *Shigella*/EIEC PCR results. 9 MHS take control measures after a positive *Shigella*/EIEC PCR result.

The numbers of reports to MHS, which are dealing with STEC PCR results, by MML varies greatly. This variation can not be accounted for by the number of inhabitants per area.

Arrangements with local MML by MHS vary greatly.

Conclusions:

1. More than 80% of MHS are dealing with STEC PCR results.

2. The number of notifications by MML to MHS varies greatly and cannot be accounted for by number of inhabitants. These differences can probably be explained by differences in indications to perform PCR on fecal samples and the fact that some MML only report culture proven STEC to their MHS.

3. Only a minority of MHS are reporting as suggested by the proposed algorithm.

4. The uniformity of reporting varies greatly leading to inadequate surveillance of STEC and *Shigella*.

5. A new laboratory algorithm has to be made and molecular diagnostics for STEC and *Shigella* have to be clinically validated.

P117

Monitoring strain diversity and dynamics in metagenomics data using meta-MLST SNP profiling

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Introduction: Microbial consortia, or complex (undefined) mixtures of microbes, are ubiquitous in nature. They are found everywhere ranging from soil to gut and from biofilms to industrial fermentations.

Monitoring bacterial diversity in consortia from meta genomics sequence data, is typically done using qPCR on strain specific genes or using 16S pyrosequencing techniques. These techniques are limited to following known strains or only allow the description of a bacterial consortium up to the genus level.

The assumption in many metagenomics studies and the subject of many published reviews is that knowing the microbial taxa composition of a metagenome also indicates the functionality

in the community. Based on the limited knowledge about individual bacterial strains and their gene content, and thus functionality, the extrapolation of knowing what species is present to metagenome functionality is therefore highly challengeable.

In addition, in many environments, e.g. from acid mine drainage, human-controlled aquatic environments and dairy starter cultures, coexistence of multiple closely related strains is observed, of which the diversity is not only determined by their unique gene content, but also by plasmid content and phage sensitivity. Our understanding of a population's complexity will therefore largely depend on the ability to differentiate between genetically highly similar individuals.

Method: We describe a method that allows following the naturally occurring bacterial diversity at the strain level by selecting 'core' genes in a set of isolated representative strains that are expected to be present in all bacterial strains present in the metagenome. Next, (combinations of) single nucleotide polymorphisms (SNPs) are determined that allow distinguishing (groups of) strains. These SNPs are subsequently used to categorize reads or contigs obtained from next generation sequence analysis of metagenomic samples into strains, similar to multi locus sequence typing (MLST). The method implies the detection of strain specific genes as well.

Results: We apply this technique to follow strain level diversity of Lactococcus lactis in multiple-timepoint meta genomics data obtained during the cheese making process. The resulting strain types are mapped on metabolic pathways.

Conclusions: We show that 1) following highly similar individual strains directly from metagenome sequence data is feasible, 2) among the isolated genomes distinct SNP patterns are maintained, even in the case of synonymous SNP's and 3) mapping of identified strain types on metabolic pathways gains insight in the metabolic potential of individual isolated strains vs. the metagenome.

P118

Staphylococcus epidermidis relocates from infected implants to the surrounding tissue in mouse experimental biomaterial-associated infection

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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*, the major cause of biomaterial associated infection (BAI), has been shown to survive inside macrophages around biomaterials implanted in mice, and was retrieved from peri-catheter tissue in humans. To study whether the tissue can become colonized starting from a biofilm of bacteria on the biomaterial surface we compared two models for BAI, i.e. with *S. epidermidis* injected along the implanted biomaterial, or deposited onto the biomaterial prior to implantation. To characterize multiple cellular immune responses in single microscopic slides of mouse tissue with both implant and bacterial infection, immunohistological staining using multiple spectral imaging was developed.
Methods: The inoculum was either injected along of the already implanted biomaterial, or applied on the biomaterial and air-dried before implantation. We compared the models at two different challenge doses of *S. epidermidis* O-47 GFP (10^5 and 10^7 CFU in 25 µl), and titanium as a biomaterial. Mice were sacrificed at 4 and 9 days after implantation, when biopsies were collected to assess the bacterial colonization of both the biomaterial and the surrounding tissue.
Multiple immunohistochemical stainings were performed on single sections. First a double staining procedure with, for example, a red chromogen for alkaline phosphatase (AP) coupled to a first antibody and a brown chromogen for horse radish peroxidase coupled to a second antibody was performed. Then a so-called HIER step, (Heat-Induced Epitope Retrieval) was performed to remove all immunoreagents from the first staining sequence, but not the deposited stains. Subsequently a second round of immune staining was performed, with 2 other chromogens. The signals of all four chromogens were unmixed with the Nuance multispectral imaging software, even when these were difficult to distinguish by the human eye. Pseudo colours were assigned to visualize the different epitopes stained by the 4 different antibodies used in the procedure.
Results: After 4 days, we cultured significantly more bacteria from the pre-inoculated biomaterial than from the biomaterial where the inoculum was injected along the biomaterial. However, the tissue surrounding the biomaterial yielded similar bacterial growth in both models. After 9 days, there is no difference between the groups, because the mice seem to clear an infection with *S. epidermidis* quite well in the presence of titanium. These results apply only to the groups with a challenge dose of 10^7 CFU in 25 µl. The groups with a challenge dose of 10^5 CFU in 25 µl showed no significant differences in any of the conditions, and were therefore not analyzed by multispectral imaging.
Using the multispectral imaging method, we were able to visualize *S. epidermidis*, macrophages and neutrophils in a single section of mouse tissue. In both mouse models, bacteria were visualized in the tissue surrounding the implant at 4 days after implantation.
Conclusion: Bacteria from a biofilm on a biomaterial surface relocate into the tissue.

P119

Escherichia coli capable of using aminoglycosides as a carbon source

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Introduction: In bacteria, resistance to aminoglycoside antibiobiotics is often due to a range of enzymatic inactivation modifying the target molecule. Phosphorylation of aminoglycoside antibiotics is a common form of inactivation in many resistant strains of *Echerichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The neomycin phosphotransferase gene nptII encodes neomycin phosphotransferase II, also called aminoglycoside 3'-phosphotransferase II (APH (3') II), which has been shown to inactivate kanamycin, neomycin, geneticin, and paromomycin by phosphorylation. Next to antibiotic resistance, a recent study found that a diverse group of bacteria, including intrinsically resistant microbes (e.g., Pseudomonadales and Burkholderiales), could subsist on and presumably catabolize antibiotics as a sole carbon source. The aim of the present study was to evaluate the capacity of *E. coli* to use aminoglycosides as a sole carbon source.
Method: The neomycin phosphotransferase gene *npt*II contained in the cloning vectors *pRSF*-1b and *TOPO* were studied in two strains of *E. coli*. Transformed and non – transformed cells were tested for their ability to subsist on the aminoglycosides kanamycin and neomycin. *E. coli DH5α* and *Top10* strains were inoculated on LB agar with and without antibiotics and grown aerobically for 16 h at 37°C prior to growth on single-carbon source (SCS) medium. SCS medium was prepared as previously described by Dantas et al, 2008, with 1, 2 and 4 mg/ml of antibiotics. One colony was picked and inoculated in SCS medium, homogenised by vortexing and then centrifuged for 5 minutes at 14000 rpm in order to prevent carry-over

of carbon source from the LB medium. The pellet was suspended and inoculated into fresh SCS medium and incubated at 370C for 24 hours, with transfers to fresh SCS for two consecutive days. In this experiment glucose (1%) was used as positive control, while SCS without any carbon sources served as negative control. Clonal isolates from the liquid cultures were obtained by plating aliquots of the cultures on LB agar media. Counting of colony forming units was performed at 0, 8, 24 and 48 hours, with all experiments in duplo.

Results: The *npt*II – transformed cells subsisted to kanamycin and neomycin at all concentrations tested, whereas non – transformed *E. coli* cells did not subsist on any antibiotic. Growth of the strains was faster in the first 24 hours and decreased at 48 hours. The growth patterns of the strains on glucose were similar to those observed in the presence of the antibiotic.

Conclusion: The neomycin phosphotransferase gene confers the ability to use aminoglycosides as a carbon source. Although previous reports indicate that antibiotic resistance and antibiotic subsistence are not equivalent, some resistance genes do facilitate both resistance and subsistence. It would be interesting to see how many more known resistance genes also facilitate subsistence, since numerous subsistence phenotypes exist.

P120

Clinical and microbiological profiles of invasive fungal infections in a non intensive-care setting in a university hospital

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Introduction: Invasive fungal infection (IFI) is a serious and increasingly frequent complication in immunocompromised patients that poses diagnostic and therapeutic dilemmas. Diagnosis is often made by a combination of indirect criteria. Antifungal therapy is available, but due to increasing resistance, immunity failing to recuperate or unrelated medical complications, the patient's condition does not always improve. The aim of this prospective observational study was to describe the types of IFI in a university hospital in a non intensive-care setting, to describe the microbiological findings and the patient population at risk as well as to determine the outcome of antifungal therapy.

Methods: From May 2011 until December 2011 clinical and microbiological data were collected on all adult, non intensive-care patients that were presented to the infectious disease consultation team and in whom treatment was started for IFI. Follow-up data were obtained 6 weeks after the start of treatment. In case of invasive pulmonary aspergillosis, diagnosis was classified according to EORTC/MSG criteria.

Results: In 28 patients treatment was started for IFI. The overall incidence of IFI was estimated as 3.0 per 1000 patient admissions in our centre (excluding paediatric and obstetric admissions).

Fourteen patients were treated for invasive candidiasis. *Candida albicans* was most frequently isolated (8/14), followed by *C. glabrata* (3/14), *C. parapsilosis* and *C. kefyr* (both 1/14). All *C. albicans* isolates were fluconazole susceptible; the *C. glabrata* isolates were all azole-resistant. All patients had a known risk factor for invasive candidiasis, such as extensive surgery or haematological disease. The crude 6 weeks mortality rate was 7%, after 6 weeks of follow-up 57% were cured, whereas the remaining 36% were still receiving treatment.

Twelve patients were treated for invasive pulmonary aspergillosis, of which 8 were diagnosed as probable and 4 as possible cases. Culture of bronchoalveolar lavage fluid or sputum was positive for Aspergillus fumigatus in only 3 patients; in one patient an azole-resistant isolate was cultured. Eight patients were treated with voriconazole only, two patients with liposomal amphotericin only and two patients were treated with more than one antifungal drug, either in combination or consecutively. Eleven patients had underlying haematological disease for which 8 had received stem cell transplantation. Crude 6 weeks mortality rate was 50%. Of the probable cases only 1 patient was cured at 6 weeks follow-up.

Conclusion: Despite advances in medical science, treatment of invasive pulmonary aspergillosis is still often started without data on susceptibility, as most moulds could not be cultured and diagnosis was therefore based on a combination of indirect criteria. The data of our study confirm previous observations that the majority of IFI consists of yeast infections in which *C. albicans* is still the most frequently found pathogen, but that in haematological patients, invasive pulmonary aspergillosis is the most frequent diagnosis. Remarkably, we found a relatively low mortality in invasive candidiasis, of which the outcome thus was more favourable compared to invasive mould infection. This is probably associated both with the underlying haematological disease as well as with the nature of invasive mould infection.

P122

Positive HIV-1 viral load results below the clinical cut-off predict viral rebound

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Introduction: It has been suggested that low-level HIV viremia, or blips during treatment are related to assay

variation and/or increased sensitivity rather than virus production. The cut-off of 50 copies/mL for virological failure is, therefore, under debate. We compared virus levels preceding blips or low-level viremia to those in a control group and determined the predictive value of viral load (VL) determinations below 50 copies/mL.

Methods: Treated patients with ongoing low-level viremia (50-5000 cp/mL, group A, n = 21) or a viral blip (group B;n = 85) were compared to a control group (n = 84) of patients with consistently suppressed virus (< 50 cp/mL) since start of HAART. Qualitative and quantitative analyses of VL determinations below 50 cp/mL in the year preceding the first detectable VL (the 'event') were performed using Roche Cobas-Amplicor v1.5 or CAP-CTM v2.0. Proportions of VL determinations by each assay were comparable among groups.

Results: Preceding the event, significantly more patients in groups A and B had RNA detected below 50 cp/ml at all time points compared to controls (60%, 20% vs 3%; p < .001). In line with these results, quantitative analysis showed higher mean VL values preceding the event in groups A and B compared to controls (10, 7 vs 2 cp/mL; p < .001). Sequence analysis revealed a new PI-resistance mutation in one of 10 tested patients of group A, suggesting viral replication in this patient. Combined analysis of all groups showed that patients who never had RNA detected in the preceding year (=TND), more often maintained a suppressed VL below 50 cp/mL compared to patients who always had RNA detected (54% vs 10%; p < .001). The occurrence of blips was lower in patients with always TND (42% vs. 55%; p < .001).

Conclusion: These results suggest that low-level viremia and blips reflect viral production or even replication, rather than assay variation. Positive VL results below 50 copies/mL predict viral rebound. We advise not to raise the currently used cut-off until there is more evidence demonstrating the clinical significance of low-level viremia and viral blips.

P123

The role of Archaea in nitrite-dependent anaerobic methane oxidation

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Two groups of microorganisms are capable of anaerobic methane oxidation: a consortium of anaerobic methanotrophic archaea (ANME) with sulfate reducing bacteria (SRB) using sulfate as the terminal electron acceptor, and NC10 phylum bacteria performing nitrite-dependent anaerobic methane oxidation (n-damo). However, in the first published n-damo enrichment, archaea of the order *Methanosarcinales* made up 10-20% of cells (Raghoebarsing, Pol et al. 2006); similar results (up to 40% very closely related *Methanosarcinales*) were obtained in an Australian enrichment culture (Hu, Zeng et al. 2009). Further studies showed that the archaeal species was dispensable for methane oxidation (Ettwig, Shima et al. 2008), but the role of the Archaea and their physiology still remained unclear. They may either be methanotrophs competing with the NC10 bacteria for nitrite/nitrate; or produce methane from metabolites of the bacteria, e.g. methanol. In this project, we use a denitrifying methane-oxidizing enrichment culture consisting of archaea (ca. 40%) and bacteria to study the potential role of these archaea. Using stable isotope labeling, the fate of methane and methanol is followed in the enrichment culture. In order to discriminate between bacterial and potentially archaeal methane oxidation, specific inhibitors (acetylene, BES) are currently tested. The results are discussed in the context of the widespread environmental occurrence of this new branch of *Methanosarcinales*.

References:

1. Ettwig KF, Shima S, et al. Denitrifying bacteria anaerobically oxidize methane in the absence of Archaea. Environ Microbiol. 2008;10:3164-73.
2. Hu S, Zeng RJ, et al. Enrichment of denitrifying anaerobic methane oxidizing microorganisms.' Environmental Microbiology Reports. 2009;1: 377-84.
3. Raghoebarsing AA, Pol A, et al. A microbial consortium couples anaerobic methane oxidation to denitrification. Nature. 2006;440:918-21.

P124

Life-cell imaging of aerobic bacteria; a tool to assess the heterogeneous germination and outgrowth of *Bacillus subtilis* spores

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Spores of various Bacillus spp. can remain in a dormant, stress resistant state for long periods. Their return to vegetative cells involves a rapid germination followed by a more extended outgrowth phase. Spore-forming bacteria are a special problem for the food industry as some of them are able to survive preservation processes. Spore germination and outgrowth progression is often very heterogeneous and therefore makes predictions of microbial stability of food products exceedingly difficult. Mechanistic detail of the cause of this heterogeneity is necessary. In order to examine heterogeneity we made a novel cast for live imaging which allows the growth, germination and outgrowth of *Bacillus subtilis* cells and spores, respectively. In order to check the efficiency of the setup, growth and division of *B. subtilis* 1A700 vegetative cells was checked at different concentrations of rich undefined media (TSB, LB), as well as a defined medium (MOPS). Phase-contrast images were

recorded every 30 s for 4 hours and doubling times were calculated. We were able to monitor nine areas in one slide per time-point using a routine that steers the lens appropriately. Thus, maximally ~100 starting cells (or spores) could be examined per experiment. The calculated generation times in our system are comparable to generation times obtained in well-aerated shake flask cultures. Hence, the setup is suitable for heterogeneity measurements at the single cell/spore level. Preliminary results show that also proper germination and outgrowth of spores is observed in our setup. To monitor where most heterogeneity ensues, recording of germination (phase bright to phase dark transition) and outgrowth times (formation of two cells) of individual spores is in progress. Current challenges are to extend the observation time from 4 hours to 24 hours such that monitoring of spore outgrowth of damaged spores as well as outgrowth under adverse conditions can be started.

P125

Comparison of Variable Number of Tandem Repeats typing and Restriction Fragment Length Polymorphism typing in the molecular epidemiology of tuberculosis in the Netherlands

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In order to switch from Restriction Fragment Length Polymorphism (RFLP) to Variable Number of Tandem Repeats (VNTR) typing of Mycobacterium tuberculosis isolates in the Netherlands, a detailed evaluation on discriminatory power and agreement with findings in source case investigation was performed on *M. tuberculosis* isolates obtained from 3,975 patients in the period of 2004-2008.

The level of discrimination between the both typing methods did not vary significantly; RFLP typing yielded 2,476 distinct patterns, compared to 2,554 by VNTR typing. Isolate type interpretation as unique or clustered in the databases remained the same for 79% (n = 3,079) of all single pattern cases (n = 3,890). For the remaining cases, 12% (n = 461) was only clustered by VNTR, 8% (n = 299) only by RFLP and 1% (n = 51) was clustered by both typing methods but showed another cluster composition.

Source case investigation was performed for 87% (n = 1,432) of the RFLP clustered cases. For cases clustered by both typing methods, 60% could be confirmed by source case investigation. For the cases clustered by RFLP but not by VNTR, the epidemiological links were only confirmed for 18%. This indicates that clustering on basis of VNTR typing has a better correlation to source case finding and contact tracing.

Samples clustered by RFLP, but split-off by VNTR typing were not associated to a particularly genotype family compared to the genotype distribution of the complete set. However, the other way around; samples that belong to a VNTR cluster and split-off by RFLP typing were associated to the Beijing, CAS and EAI family. These three genotype families were two-times more represented in the group of split-offs compared to the complete set of samples.

The turn-around-time of VNTR typing was approximately 14 days after arrival of the culture at the TB reference laboratory and this is significantly faster than the average 44 days previously needed for RFLP typing.

We conclude that VNTR typing does in practice provides a speed up in typing and seems in better agreement with findings in contact tracing than RFLP typing. Next step will be to utilize this faster epidemiological typing in supporting contact tracing.

P126

A case of *Scedosporium prolificans* infection in a haematologic patient with respiratory problems

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Introduction: *Scedosporium prolificans* infections are rare, but associated with a high mortality (47%-100%), especially in patients with a heamatologic malignancy. The fungus is known to be highly resistant *in vitro* to all clinically used antimycotic agents. In addition, administration of these agents does not seem to improve survival. However, several combinations of agents have shown effect *in vitro* and in mouse models for infection. Here, we describe a case of a patient with a disseminated *S. prolificans* infection, treated with a combination of voriconazole, terbanifine and G-CSF.

Case description: In August 2011 a 68-year old male was admitted to our hospital for evaluation of abnormal liver biochemical tests. He had a history of localized melanoma, heart failure, and an allogeneic stem cell transplantation for chronic myelomonocytic leukemia, 10 months before admission, complicated by chronic graft vs. host disease for which he was treated with high dose steroids. On

admission, he was clinically well, though he complained of fatigue, peripheral oedema and frequent hematomas. He was treated with valganciclovir for a reactivation of cytomegalovirus and received itraconazole prophylaxis. A liver biopsy revealed iron overload, most probably due to repeated blood transfusions. During admission, the patient became increasingly dyspnoeic and eventually, pneumonia was suspected. A CT scan at day 22 after admission showed pleural effusion and atelectasis of the left lower lobe. Bronchoalveolar lavage (BAL) was performed and microscopy suggested infection, therefore, at day 26, empirical treatment with piperacillin/tazobactam was initiated. However, bacterial cultures, mycobacterial PCR, PCP-PCR and viral PCRs of the BAL were negative. The patient's clinical condition, especially his respiratory state, gradually deteriorated. On day 43, fungal culture of the BAL became positive with *S. prolificans* and treatment with voriconazole (6 mg/kg twice daily) was initiated. On day 44, blood cultures of day 40 and 41 became positive for *S. prolificans* as well. Because patient did not show respons to therapy, terbinafine (250 mg once daily) and G-CSF (30x106 IE once daily) were added on day 45. However, patient developed septic shock and died 46 days after admission.

Discussion: Since *S. prolificans* infections are relatively rare, no trials on treatment have been published. Case reports on *S.prolificans* are increasingly published and show a high mortality, regardless of antifungal therapy. Risk factors for mortality among patients with *S. prolifecans* infection include disseminated infection, malignancy and neutropenia. *In vitro* and *in vivo* animal studies show promising results for combination therapy of voriconazole with terbinafine, itraconazole with terbinafine, micafungin with voriconazole or micafungin with amphotericin B. The addition of G-CSF to the antifungal therapy yields controversial results. In practice, even patients receiving combination therapy only rarely survive disseminated infection. This was illustrated in our patient, who received maximal treatment with a delay of at least 23 days after the first signs of pulmonary infection. Consequently, it is possible that earlier detection and treatment might have improved his chance of survival.

Conclusion: This case report demonstrates the need for earlier detection and knowledge of the optimal treatment for *S. prolificans* infections.

P127

Molecular analysis of bloodstream infections: towards blood culture sensitivity

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Introduction: Fast Microbial diagnosis of patients with sepsis is essential to improve prognosis and to reduce the use of broad-spectrum antibiotics. Various molecular techniques have been evaluated but do not provide adequate sensitivity in clinical practice. The low volume of blood used and inhibiting factors in blood as leukocyte DNA are considered important determinants of the relatively low clinical sensitivity. In this study we determine the influence of increased blood volumes on clinical sensitivity of bacterial DNA detection in blood from patients with proven bloodstream infection. For this purpose we used a new rapid method (Polaris®) that is in development within Biocartis which claims to selectively remove human DNA prior to the extraction of bacterial DNA enabling higher blood volume input in the PCR. Preclinical results revealed a sensitivity of up to 1 CFUeq/ml but have to be confirmed in clinical specimens. In this pilot study we compared this new method with a reference test for detection of DNA in blood samples from patients with bacteraemia.

Methods: Whole blood samples (6ml EDTA) from 9 patients with blood cultures positive for *Enterococcus faecalis* (n = 3) or *Staphylococcus aureus* (n = 6) were analysed. Samples were processed using the Polaris method (5ml) and the reference method (0,2ml). The reference method achieves bacterial lysis with Triton-Tris-EDTA for *E. faecalis* and lysostaphine for *S. aureus*. The Polaris method is based on non-enzymatic cell lysis. For both methods DNA was extracted with the EasyMag® system and quantified with a specific real-time PCR assay (Lightcycler 480®). Additionally, serial measurements during treatment for culture-proven endocarditis were performed in three patients.

Results: Bacterial DNA was detected in 8/9 patients with the Polaris method and in 6/9 with the reference method; the signal in the other samples did not cross the detection limit. The Polaris method had a median cycle threshold (CT) value of 35.2 (range 29.7 - > 42) compared to 36.7 (range 32.7 - >42) with the reference method. A Wilcoxon signed rank test showed a significant difference between both methods (p = 0.04). Bacterial DNA remained longer detectable with Polaris than with the reference method in serial blood samples of patients with endocarditis. With the Polaris method the bacterial load was detectable for at least 6 days in all three patients. A bacterial DNA load of less than 10 CFUeq/ml was detectable in clinical specimen using the Polaris method.

Conclusion:

1. The Polaris method has a higher sensitivity than the reference method in clinical samples and provides more

possibilities for serial measurements in clinical blood specimen during treatment.
2. The larger volume that can be processed with this method is likely an important factor for clinical usage. This method may provide the step that increases sensitivity to the level of blood culturing and makes the molecular detection of bloodstream infections clinically applicable.

P128

***Echinococcus multilocularis* as emerging infection in the Netherlands**

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The fox tapeworm, *Echinococcus multilocularis*, is endemic in large parts of Europe. The endemic region for *E. multilocularis* in foxes appears to be spreading toward the north and now includes south-eastern parts of the Netherlands, specifically Limburg and north-eastern parts of Groningen (1). The final host of *E. multilocularis* are foxes or dogs and usually the intermediate hosts, where the eggs hatch and form larvae that reside in the body, are rodents. When humans become an accidental intermediate host it will lead to serious disease known as alveolar echinococcosis. Starting in the liver multiple cysts may develop in various organs. When left untreated the disease can be lethal.
Patients, suspected of echinococcosis, can be diagnosed in various ways. In our laboratory we perform diagnosis serologically or molecularly. The molecular approach requires cyst fluid or a biopsy of the cyst wall in order to retrieve DNA and the species of cestode can be determined. We used to detect and type all cestodes using a PCR on the Cox1 and Nad1 genes. These two markers proved to be sometimes problematic because of variant nucleotides at the primer annealing sites. We, therefore, compared various alternative markers with sufficiently conserved primer annealing sites. Recently, we have extended the detection and typing of Echinococcus spp. with PCRs on the 12S (2) and Nad5 genes. These genes have proven to be slightly more sensitive than the previous markers and also show somewhat more genetic diversity.
In 2008 we found a patient positive for *E. multilocularis* using PCR. This patient, who lives in the south-eastern part of the Netherlands has probably been infected in the Netherlands. In the autumn of 2011 we found two other patients that have an *E. multilocularis* infection. One of these is probably infected in the Netherlands, the other is unknown. We need to extent our genetic analysis with more markers if we want to compare the DNA sequence of Dutch *E. multilocularis* of foxes with those found in Dutch patients and thus resolve the matter with a molecular approach.

P129

***In vitro* botulism test development with C- and D- toxin serotype specificities**

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Introduction: The neurotoxins of *Clostridium botulinum* are considered as the most toxic biological agents in nature. Although botulism occurs worldwide, disease in humans is rare. Outbreaks among animals occur more frequently, but are usually not related to human cases. Not only diagnosis of clinical cases, but also environmental occurrence of bacterial spores and risks of misuse in bio-terrorism like activities, requires continuous alertness and testing.
Botulinum neurotoxins (BoNT) inhibit neurotransmitter release at the neuromuscular junction. Specifically, the proteolytic activity of these toxins is targeted on SNARE (Soluble NSF Attachment Receptor) proteins which are involved in fusing synaptic vesicles to the synaptal plasma membrane, followed by release of acetylcholine at the neuromuscular junction. Seven BoNT types are described (BoNT A-F). Each type is antigenically distinct. The types A, B and E (rarely F) cause human disease, while types B, C, D and E are the main toxins causing disease in animals. To date the mouse bioassay is the golden standard in laboratory diagnosis. In the Netherlands these are routinely carried out by the Central Veterinary Institute (CVI), both for veterinary and for medical diagnostic samples. There is a need for *in vitro* alternatives, however these are not yet generally available and accepted. Main problems in the development of *in vitro* tests have been the high sensitivity that is required and the need to cover the whole range of toxin types (BoNT A – F).
Research developments towards such assays show that the proteolytic specificities of the toxins are closely linked to their antigenicity and thus can be used to improve the sensitivity and specificity of *in vitro*-tests. Goal of this study is to show proof of principle to detect BoNT/C and D using the proteolytic cleavage specificity for their biological SNARE complex proteins, in combination with a novel capture system using brain microsomes containing natural toxin receptors. In a current study, these capture conditions are being investigated and the potential to use these complexes for toxin detection.
Methods: Immunochemical tests have been developed for monitoring proteolytic toxin activity, using SNARE protein based synthetic peptides as substrate. Conditions for BoNT/C and BoNT/D capture by brain vesicles have been established using different animal species as tissue source.
Results: Conditions for BoNT/C and /D capture by brain vesicles have been succesfully established. Different animal species as tissue source could be used.

Immunochemical assays yielded specific detection of BoNT/C both as free toxin and in microsome bound condition.

Conclusions: The results point out that:

1. Brain microsomal fractions allow BoNT/C and BoNT/D capture.
2. Proteolytic activity detection of free and captured BoNT/C is currently in the range of 10 mouse lethal doses and will be further improved in the optimisation phase of the assay.
3. Toxin capture systems coupled to sensitive and specific proteolysis based *in vitro* tests are promising assay systems and – based on ethical considerations – highly preferred above the mouse bioassay.

P130

Presence of *Chlamydia psittaci* in birds in the Netherlands; results of diagnostic testing at CVI from 2006 to 2011

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Introduction: Psittacosis is a zoonosis, caused by the obligate intracellular agent *Chlamydia psittaci*. Although infection may occur without disease, pittacosis in humans is characterised by mild to severe flue like symptoms including fever, headache, muscle aches, cough, chills and sweating. In severe cases it may lead to pneumonia or septic syndrome with multi-organ failure for which hospitalization is necessary.

Birds are considered to be the main reservoir of the bacterium for human infection. Inhalation of dried, contaminated excreta from birds is considered the most important infection route.

A broad range of bird species have been shown to be a suitable host for *C. psittaci*. In birds *C. psittaci* can lead to clinical signs of disease (conjunctivitis, inactivity and weight loss), but often animals are symptomless carriers. Birds can excrete *C. psittaci* intermittently for years and short-term contact with *C. psittaci* secreting birds or their excreta is probably sufficient to cause infection.

It has been suggested that psittacosis may be underdiagnosed because of the highly variable presentation of infection with *C. psittaci*. Data on the prevalence in Dutch bird species can be valuable information for clinicians and medical microbiologists in the Netherlands to estimate the risk for psittacosis in their patients.

Methods: During 2006 to 2011 samples from 6603 animals were tested at the Central Veterinary Institute (CVI). The majority of samples consisted of cloacal swabs or fecal samples originating from across the Netherlands which were sent to CVI for testing the presence of *C. psittaci*. Most samples were submitted either because of a suspicion of avian chlamydiosis or for the purpose of source tracing of human cases, meeting Dutch regulations on psittacosis.

After DNA extraction, samples were tested in a real time Polymerase Chain Reaction (PCR) targeting the *OmpA* gene.

Results: Over the years 2006 to 2011, 6603 animals have been tested. Overall 3.0% of the samples were positive for *C. psittaci*, varying from 1.8% 2011 to 5.7% in 2006.

Submissions consisted either of single samples from an individual animal or multiple samples from a bird population or flock. On average, submissions involved 4.4 sampled animals, ranging from 1 to 195. Generally, 7.8% of the submissions contained one or more positive samples. When multiple samples were collected from a population or flock, the median positive percentage of samples was 25.0% (ranging from 1.6% to 100%).

As expected, highest prevalence was detected in samples from pigeons and psittacine birds (parrots, cockatoos and parakeets) with 5.1% and 4.0% of tested samples positive for the presence of *C. psittaci* respectively. *C. psittaci* was also regularly identified in samples from various wild bird species (3.1%), in 0.8% of poultry samples, but in none of the samples from songbirds (canaries).

Conclusions:

1. These data suggest that the overall infection rate of birds in the Netherlands is low.
2. In positive populations or flocks, a considerable part of the animals may excrete *C. psittaci*.
3. Highest prevalences were found in samples from pigeons and psittacine birds.

P131

Low performance of rapid latex agglutination test to identify *Clostridium difficile* in routine diagnostics.

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Background: Phenotypical identification of *Clostridium difficile* is cumbersome and time consuming. Therefore, a rapid agglutination test has been developed (M41 Microgen *Clostridium difficile*, Microgen bioproducts, Surrey, UK). The principle of the test is macroscopical agglutination of bacteria with latex particles coated with rabbit IgG antibodies specific for C.difficile cell wall antigens. This test is intended for confirmatory identification of C.difficile cultured on solid media.

Aim of the study: To determine the performance of a rapid agglutination test for *C. difficile* in comparison with a gold standard for identification of *C. difficile*.

Methods: In a prospective setting at the National Reference Laboratory for *C. difficile*, all colonies suspected for *C. difficile* were tested by rapid agglutination. As gold standard, the presence of *C. difficile* specific glutamate dehydrogenase (GDH) was investigated by a home-made PCR. Colonies with discrepant results were further investigated by Maldi-Tof MS. All *C. difficile* isolates were further characterized by PCR ribotyping.
Results: From January 2011 till December 2011 we tested 227 colonies from submitted samples. All tested colonies had a typical morphology of *C. difficile* on selective media. Compared to the presence of GDH, sensitivity, specificity, positive predictive value and negative predictive value of the agglutination test was 86%, 75%, 91% and 64%, respectively. *C. difficile* isolates false negative tested by agglutination samples (n = 15) belonged to 10 different PCR ribotypes. Available isolates false positive tested (n = 14) were further investigated and were identified as Clostridium bifermentans, Clostridium clostridioforme, Clostridium hatheway, Citrobacter freundii and Lactobacillus sakei. Only Clostridium bifermentans is known to have cross-reactive cell wall antigens with *C. difficile*.
Conclusion: The performance of a rapid agglutination test to identify *C. difficile* is too low to recommend this test for application in routine diagnostics.

P132

Twin small non-coding RNA molecules involved in feast/famine regulation in *Neisseria meningitidis*

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Introduction: Small non-coding RNA molecules (sRNAs) are present across the genome of prokaryotes. They are involved in riboregulation where they function as quick and economical regulators of protein expression through interaction with mRNA molecules. This interaction of sRNAs with their mRNA targets can be enhanced by Hfq, a chaperone protein which has the ability to stabilize these complexes and influence degradation. The obligate human bacterial pathogen *Neisseria meningitidis* is capable of causing invasive disease characterized by life-threatening septicemia and meningitis. In developed European countries such as the Netherlands, meningococcal disease remains a threat due to the lack of a vaccine against serogroup B strains. The aim of this study is to discover and characterize sRNAs of this pathogenic species in order to better understand its physiology which might open new paths to future therapeutic options.
Methods: Bioinformatic prediction and confirmation by RNA-sequencing were used to detect the presence and expression levels of sRNAs in N. meningitidis. Putative targets of discovered sRNAs were consequently predicted in silico. These potential sRNA-mRNA interactions and their effect on protein expression were assessed using a gfp reporter system for translational control and target recognition of sRNAs in *Escherichia coli*. Furthermore, sRNA deletion and overexpression mutants of N. meningitidis were created and proteomic/mass spectrometry analyses were performed to identify differentially expressed proteins. Growth curves were determined in rich and carbohydrate limited medium.
Results: Investigation into the existence and function of sRNAs in N. meningitidis revealed two structurally nearly identical sRNAs located in their immediate vicinity, with 70% sequence identity (twin sRNAs). A twin sRNA deletion mutant showed differential expression of seven proteins. Four of these proteins are directly involved in the tricarboxylic acid (TCA) cycle: AcnB, GltA, Icd, and SucC. All four were previously shown to be regulated by Hfq. Direct translational control by one of the twin sRNAs was proven for mRNAs encoding the TCA cycle enzymes GltA and SdhC, and for PrpB; an enzyme involved in metabolism of Succinyl-CoA, a carbohydrate constituent of the TCA cycle. Constitutive overexpression of twin sRNAs does not impair growth in nutrient-rich medium, whilst growth was drastically inhibited in medium with glucose as the only source of carbohydrate.
Conclusions: Twin sRNAs in N. meningitidis is an inhibitor of the TCA cycle during logarithmic growth in rich medium (feast). Overexpression of twin sRNAs in meningococci growing in poor medium (famine) inhibits growth due to inhibition of the TCA cycle. This is the first example of a sRNA involved in regulation of protein expression in bacteria switching between feast/famine growth conditions. We propose to name this sRNA feast/famine regulator RNA (FfrR).

P133

Multicenter evaluation of a novel non-enzymatic method for enrichment of pathogen DNA from large volumes of whole blood

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Sensitive molecular analysis of bloodstream infections using large sample volumes is hampered by the excess of leukocyte DNA in blood that inhibits the PCR.
In this study we evaluated a novel non-enzymatic enrichment method for the selective isolation of DNA from

pathogens present in 1-5 ml EDTA blood with efficient removal of human DNA.
The method was evaluated by testing healthy donor blood spiked with 1-1000 cfu/ml of *S. aureus, P. aeruginosa* and *C. albicans* with species-specific rtPCR assays. The method was compared with a DNA extraction method optimized for 200 µl blood (EasyMAG) and a commercial enzymatic procedure for selective enrichment from 1-5 ml blood (MolYsis Complete 5 kit, Molzym).
Increasing sample volumes from 200 µl using the EasyMag method to 1, 3 and 5 ml with the non-enzymatic enrichment method reproducibly resulted in concurrent lower Ct values and higher detection levels at borderline concentrations (e.g. 40-50% and 97-100% positive at 10 cfu/ml with 200 µl EasyMAG and 5 ml enriched samples, respectively). Similar Ct values and detection rates were obtained after enrichment of 5 ml blood with the enzymatic method (Molzym) and non-enzymatic method, but the latter method was much faster, less labor intensive and more reproducible. Tested pathogens were detectable at concentrations of 1-10 cfu/ml blood.
In conclusion, the novel enrichment method enables the reliable detection of bacteria and fungi at clinically relevant concentrations by standard molecular tests within 2 hours. Automation of this assay is currently ongoing and will facilitate clinical studies.

P134

Use of lytic bacteriophages to reduce the number of ESBL producing *E. coli* on chicken skin

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Introduction: bacteriophages (phages) are considered as possible biological alternatives to antimicrobials used in the food industry to decontaminate the surfaces of consumables. The main advantage of phages is their safety, because they do not cause harm to the food or consumer. Still, only for some food pathogens, such as Listeria, efficacy of lytic phages in reducing bacterial contamination gave rise to the development of a practical application. Some of the drawbacks of decontamination with phages are namely limited host range or phage-resistant mutants. Therefore the use of broad host-range phages or a cocktail of multiple phages with different specificities are considered as preferred strategy.
Aim: This study was conducted to investigate the feasibility whether a set of coli-phages could be isolated, which were able to kill a representative selection of ESBL producing *E. coli* strains from Dutch poultry.
Methods: a representative selection of 50 different ESBL producing *E. coli* strains from Dutch poultry was established comprising different O:H-serotypes and ESBL genes. Wastewater from a major chicken slaughterhouse served as the main source for isolation of coli-phages. Phages were detected, isolated and subsequently enumerated according to classic bacteriological methods.
Chicken breast skins were subjected to phage treatment by submersion in a phage cocktail (1.0E+07 pfu/ml). Bacterial counts before and after were recorded.
Results: for all 50 selected *E. coli* strains lytic bacteriophages could be isolated at the first attempt. The host-range of phages varied between 20 and 90%. A cocktail of 10 phages was able to kill all strains. Highly contaminated chicken breast skins could be reduced by 7 log cfu/g whereas low contaminated skin by only a 2-log reduction.
Conclusions:
1. Lytic phages for ESBL producing *E. coli* strains from Dutch poultry are easily isolated.
2. Decontamination of chicken skin to reduce the number of ESBLs can be achieved by submersion in a lytic coli-phage cocktail.

P135

Use of PCR-assay directly on nasal swabs for a rapid detection of high-level mupirocin resistance

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Introduction: Eradication of *Staphylococcus aureus* colonization with nasal mupirocin-ointment is an effective strategy to prevent staphylococcal infection and transmission. Abundant use of mupirocin has been reported to cause emergence of mupirocin resistance. Increase of high-level mupirocin resistance (MIC ≥ 512 ug/mL) is an important clinical problem since high-level mupirocin resistance is associated with decolonization failure.
In our hospital an increase in high-level mupirocin resistance has been seen in coagulase-negative staphylococci (CNS). High-level mupirocin resistance is mediated by a plasmid-encoded *ileS-2* gene, and can be transferred to other Staphylococcal species including *S. aureus*. Therefore, screening for high-level mupirocin resistance might be considered if reliable rapid testing would be available.
Aim: To develop and validate a real-time PCR assay for the detection of the *ileS-2* gene.
Methods: We assessed bloodstream CNS isolates collected from 2006-2011 to validate real-time PCR on bacterial colonies. Mupirocin susceptibility was tested by Phoenix automated testing and Etest confirmation. Nasal swabs were prospectively collected in December 2011 and streaked on plates for detection of mupirocin resistant colonies and then used for DNA isolation. Growth on agar plates containing 8 ug/mL mupirocin with MIC value determination by Etest was used as gold standard for

detection of high-level mupirocin resistance. In case of bacterial growth, bacterial identification was performed by MALDI-TOF. PCR primers and probe set were designed from an alignment of available *ileS-2* gene regions that were both specific for and conserved within Staphylococcal *ileS-2* sequences. Resulting primer/probe was tested against DNA from a set of bacterial species/isolates with known mupirocin resistance and the DNA extracted from the nasal swabs.

Results: When tested against isolates with known resistance *IleS-2* RT-PCR was positive in 81/82 mupirocin high-level resistant CNS isolates, and negative in 513/516 susceptible or low-level resistant CNS isolates (sensitivity 0.99, specificity 0.99). When testing 47 freshly collected swabs, the bacteria growing on mupirocin plates represented mainly *Corynebacterium* species which are known to be mupirocin resistant. For one swab also a few *Staphylococcus epidermidis* colonies (MIC> 1024 ug/mL) grew on the corresponding plate.

PCR on the DNA isolated from the swabs was positive (Cq < 35) for 4/47 nasal swabs (9%). The sample with the highest *IleS-2* DNA load (Cq 31.0) corresponded with the agar that displayed growth of mupirocin resistant *Staphylococcus epidermidis*. The other 3 PCR positive samples had a Cq indicative of a lower *IleS-2* content (Cq 33.8, 34.2 and 34.8, respectively). No mupirocin resistant Staphylococci could be detected on the corresponding mupirocin plates, despite the fact that one sample (Cq 34.2) was from a patient known to carry mupirocin resistant CNS prior to sampling.

Conclusions: This real-time PCR assay offers a rapid and specific method for detection of high-level mupirocin resistance on nasal swabs. The real-time PCR might be more sensitive for high-level mupirocin resistance detection than culture, since in one case of a known carrier PCR was positive and culture negative.

P136

Detection and molecular characterization of methicillin-resistant *Staphylococcus aureus* carrying SCCmec XI with a novel mecA homologue in humans in the Dutch-German Euregio

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Introduction: Since 2005 the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in livestock focused attention. Very recently the situation has been dramatically complicated by the emergence of bovine and human isolates carrying a new staphylococcal cassette chromosome mec (SCCmec) element (type XI) leading to phenotypic beta-lactam resistance. This SCCmec element contains a novel mecA homologue, designated mecALGA251, which is not detectable by conventional mecA-specific PCR approaches and PBP2a agglutination tests. Identified mecALGA251 positive strains belonged to distinct clonal lineages (CC130 and ST425). Here we report on 5 MRSA isolates from 3 distinct clonal lineages containing novel mecA homologue mecALGA251 recovered from patients in the Dutch-German Euregio.

Methods: A total of 236 non-repetitive MRSA isolates recovered from patients in the hospitals located in the Dutch-German Euregio between 2003 and 2010 were analyzed in the current study. All isolates in the collection were characterized by spa typing. The isolates phenotypically PBP 2a positive but lacking mecA by conventional DNA microarray screening were further tested by non-commercially available SCCmec XI microarray probes (Alere Technologies GmbH, Jena, Germany). Those isolates which carried novel mecA homologue mecALGA251 were subsequently characterized by multilocus sequence typing (MLST). The virulence genes were detected by the commercially available microarrays (StaphyType by Alere Technologies GmbH, Jena, Germany).

Results: Among the tested isolates we found 5 isolates carrying the SCCmec XI with the mecA homologue mecALGA251. Three of the five isolates belonged to MRSA CC130 by MLST with the spa types t843, t1535 and t1773, whereas the remaining 2 isolates were representatives of MLST Sequence Types (STs) which were not already described for the presence of SCCmec XI, ST599 (with spa type t5930) and ST1946 (with spa type t978). The isolates with SCCmec XI were recovered from the patients between June 2009 and May 2010. Almost all isolates were recovered from nose swabs of asymptomatic carriers with the exception of a single isolate with spa type t843 which was recovered from skin infection. Isolates of MLST CC130 did not carry any virulence gene with the exception of a single isolate (with spa type t843) which harbored the sec gene. In contrast, new identified MRSA genotypes containing the SCCmec XI were characterized by the presence of higher number of toxin genes: ST599 isolate possessed 3 toxin genes (tst1, sec, and sel), while ST1946 isolate had 8 toxin genes (tst1, sec, seg, sei, sel, sen, seo and seu).

Conclusions: The current study showed acquisition of SCCmec XI with mecALGA251 to next *S. aureus* genotypes, which complicates the identification of MRSA. Thus, DNA-based MRSA rapid detection assays targeting this gene sequence would fail to detect these strains. Culture-based detection needs to be performed in parallel to molecular detection. Furthermore, most of the MRSA with SCCmec XI carry no tested virulence factors, some of them

harbor many important virulence factors which show the genomic plasticity of MRSA.

P137

Evaluation of the efficacy of an autogenous whole bacterin *Streptococcus suis* serotype 9 vaccine in pigs

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Introduction: In intensively raised production animals vaccination is an important tool in on-farm disease management. Vaccination helps to limit the preventive and therapeutic usage of antimicrobials. However, for some bacterial animal diseases effective licensed vaccines are still not available. When no approved vaccine is available, it is allowed by law and under strict conditions, to prepare and apply autogenous whole bacterin vaccines. These vaccines are based on inactivated farm-specific isolates from clinical cases. For the control of *Streptococcus suis* (*S. suis*) infections in pigs, these autogenous vaccines are widely used in the Netherlands. *S. suis* is an important pathogen in pigs worldwide, causing arthritis, meningitis, endocarditis or sudden death in young pigs. *S. suis* can also cause severe infections in humans. Of the 33 known capsular serotypes of *S. suis*, serotypes 2 and 9 are worldwide most often associated with clinical signs in commercial pig farms. Serotype 9 is responsible for most cases in the Netherlands. Although widely used, the efficacy of autogenous *S. suis* vaccination against serotype 9 infections is, however, unknown. The aim of our study was to determine the effect of vaccination with an autogenous whole bacterin vaccine containing *S. suis* serotype 9 on mucosal colonization, on clinical signs, and on transmission of this serotype among pigs after homologous challenge.
Materials and methods: Animal experiments were performed with caesarean derived, colostrum deprived pigs (n = 50), housed pair-wise. Thirteen pairs were intramuscularly vaccinated at 3 and 5 weeks of age with 2-3[GREEKX]10^9 colony forming units formalin inactivated *S. suis* serotype 9 and α-tocopherolactetaat as adjuvant. Twelve pairs served as non-vaccinated controls. At 7 weeks, one pig of each pair was intranasally inoculated with 1-2[GREEKX]10^9 colony forming units (CFU) of the homologues strain; the other pig was contact exposed. Saliva swabs and tonsil brushings were collected for 4 weeks, and tested for the presence of *S. suis* by quantitative bacteriological culture. In the same period clinical scoring was performed daily. Blood samples taken at 3 and 7 weeks were tested for S. suis serotype 9 specific IgG in an indirect ELISA.
Results and discussion: Despite specific systemic IgG antibody responses in vaccinated pigs, no differences in quantity of *S. suis* in tonsilar or saliva samples were observed between vaccinated an control pigs. Mean values in vaccinated pigs were $2.71x10^5$ and $1.18x10^6$ CFU per saliva or tonsil sample, respectively. In control pigs this was 2.39 $x10^5$ and 0.73 $x10^6$ CFU for saliva (P = 0.99) or tonsil (P = 0.95), respectively. In all pairs, transmission between inoculated and contact exposed pigs occurred, and no difference was observed in transmission rate between the groups. The estimated transmission rate parameter β was 5.27/day among vaccinated pigs, and among non-vaccinated pigs 2.77/day (P = 0.18). No differences in clinical signs were observed between vaccinated and control pigs, which is in contrast to published *S. suis* serotype 2 studies.
Conclusion: Vaccination against *S. suis* serotype 9 under experimental conditions did not reduce transmission, nor colonization and there were no indications that protection against clinical signs was induced.

P138

Genetic requirement for *Moraxella catarrhalis* serum resistance

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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. When colonizing the hosts mucosal surfaces, *M. catarrhalis* faces the challenge of resisting the innate immune system, including the complement system. Several molecular epidemiological studies have demonstrated that the *M. catarrhalis* species is composed of two phylogenetic lineages, referred to as the serosensitive and seroresistant lineage, the latter being enriched for strains that are resistant to complement-mediated killing. Importantly, almost all *M. catarrhalis* isolates from diseased individuals are resistant to the effects of normal human serum. To gain more insight into the molecular basis of *M. catarrhalis* complement-resistance, we have used the genomic array footprinting (GAF) technology, a high throughput genome-wide negative selection screen, to identify genes that are essential for survival of *M. catarrhalis* during exposure to human serum.
Methods: For the serum GAF screen, a mariner transposon mutant library (~7,000 mutants) of *M. catarrhalis* RH4, a complement-resistant isolate from the seroresistant-lineage, was challenged with 40% normal human serum (NHS;

challenge) or heat-inactivated NHS (control). Chromosomal DNA from both conditions was extracted and subsequently used to generate mutant-specific DNA probes. Mutants with increased susceptibility to NHS were identified by differential hybridization of mutant-specific probes from control and challenge conditions to microarrays. For target validation, gene deletion mutants were generated and tested individually for their complement-sensitivity. Gene distribution and conservation was analyzed using the publicly available genome sequences of seroresistant lineage isolates and seven newly sequenced serosensitive lineage isolates.

Results: Our genome-wide screen identified 76 genes that appeared to play a role in complement resistance of *M. catarrhalis*, as the corresponding transposon mutants were negatively selected from the library during exposure to NHS. In accordance with previous studies, five of the identified genes are directly linked to the biosynthesis of lipooligosaccharide (LOS). Further, we identified several novel genes that appeared to be involved in serum resistance, among which genes encoding the peptidoglycan recycling protein Mpl and the disulfide bond formation protein DsbB, and 21 genes that are either annotated as (conserved) hypothetical or of unknown function. Importantly, we confirmed the role of *mpl* and *dsbB* in seroresistance of *M. catarrhalis* using directed gene deletion mutants. Interestingly, the newly identified genes were found to be present in isolates of both phylogenetic lineages, with only limited sequence variability.

Conclusions: To increase our understanding of the genetic requirement for *M. catarrhalis* serum resistance we successfully screened a large transposon mutant library using GAF technology. In addition to the LOS structure, we found several novel genes that play an important role in complement-resistance of *M. catarrhalis*. Currently, the specific roles of the identified genes in serum resistance of *M. catarrhalis* are under investigation.

P139

Delimitation and characterization of *Talaromyces purpurogenus*

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According the concepts of single name nomenclature, all accepted species of Penicillium subgenus Biverticillium are transferred to Talaromyces by Samson et al. (2011). Species of biotechnological and medical importance, such as *P. puropurogenum, P. funiculosum, P. marneffei*, are now combined in Talaromyces. *Talaromyces purpurogenus* is characterized by symmetrically biverticillate branched conidiophores with lanceolate phialides. The production of intense rapidly diffusing red pigmentation on Czapek Yeast agar at 25°C is one of the most striking features of this species. It is common in tropical and subtropical regions and has been isolated from tropical cereals, fruits and nuts and produces a variety of biotechnologically interesting enzymes and pigments. It has been isolated from clinical specimens. Identification of *T. purpurogenus* strains remains difficult and most of our present knowledge is based on morphological data. In this study, isolates of *T. purpurogenus* and related species were studied using molecular techniques (sequencing the ITS regions, parts of the β-tubulin and RPB1), macro- and micromorphology features, and extrolite profile patterns. Isolates belonging to the *P. purpurogenum*-complex were cultured according to the methods descriobed by Frisvad and Samson (2004). The secondary metabolites were analysed using the agar plug method. The representative strains, including the ex type cultures of all species were examined by high-performance liquid chromatography (HPLC) with diode array detection. Also additional strains were investigated for the secondary metabolite patterns. Our molecular data indicate that the type cultures of *T. purpurogenus, P. crateriforme* and P. sanguineum are similar and therefore *P. crateriforme* and P. sanguineum are placed in synonymy with *T. purpurogenus*. Since *P. crateriforme* is placed in synonymy with *T. purpurogenus*, the mycotoxins rubratoxin A and B, previously claimed to be produced by *P. crateriforme*, are linked to *T. purpurogenus*. Based on our molecular data, the majority of the strains previously identified as *T. purpurogenus* can be subdivided into three groups. Each group has distinct micro- and macromorphological features and a unique extrolite pattern.

P140

Analysis of the *B. subtilis* membrane proteome of a strain deficient in *Bacillus disulphide* bond proteins

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Introduction: Oxidative protein folding, specifically the formation of disulphide bonds, is essential for the correct structure and activity of a number of secreted and membrane-associated proteins. *Bacillus subtilis* is a gram-positive model organism with Generally Regarded as Safe (GRAS) status. It is often used in industrial settings for protein production as it is easily genetically manipulated and able to produce high yields of secreted proteins. Oxidative protein folding in bacteria is performed by thiol-disulphide oxidoreductases (TDOR) and in *B. subtilis* TDORs are referred to as Bacillus Disulphide Bond proteins (Bdb). Four Bdb proteins have been identified in

B. subtilis: BdbA, B, C and D. The gene for these proteins are grouped in pairs on the genome: *bdb*A and *bdb*B are found in the Spβ prophage region and *bdb*C and *bdb*D are operonic. BdbC and BdbD are the native Bdb proteins of *B. subtilis* and form a redox pair important for the correct folding of the competence protein ComGC, while BdbB and BdbD are associated with the correct folding of Spβ prophage-associated antibiotic sublancin 168. Apart from these two substrates no further native Bdb substrates have been identified.

Methods: In this study we analysed specifically the membrane proteome of a *Bdb*CD mutant strain. *B. subtilis* is known have high proteolytic activity with a large number of extracellular proteases, hence the absence or changes between the *B. subtilis* and mutant strain could suggest potential direct or indirect Bdb relationships. The membrane proteomes of the *B. subtilis* 168 and a *B. subtilis* fjBdbCD mutant strain were compared. Membrane proteins were enriched for and extracted by cellular fractionation techniques and tryptically digested directly in-solution. Peptides were separated and identified by extended liquid chromatography coupled to mass spectrometry (LC-MS/MS). Two separate membrane protein extractions were performed which generated two biological replicate experiments. These biological replicates were injected three times to generate three technical replicates.

Results: The total numbers of proteins identified in the *bdb*CD mutant and the parental strains were comparable. However 15 proteins were absent in the mutant strain and 3 suddenly present. The proteins that were absent from the mutant strain are candidate substrates for oxidative folding by BdbCD, and the newly appearing proteins may belong to compensatory mechanisms that are activated due to the absence of BdbCD.

Conclusion: By using different membrane proteomics approaches a number of novel potential TDOR substrates, proteins associated with TDOR substrates or compensatory mechanisms related to the loss of BdbC and BdbD were identified in this study.

P141

Immunoglobulin subclass switching due to exposure to *Staphylococcus aureus*

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Introduction: The human pathogen *Staphylococcus aureus* is consistently carried in the anterior nares of 30% of the human population and almost 60% of the human population intermittently carries this pathogen. *S. aureus* causes diseases, varying from skin and soft tissue infections to deep seated pneumonia and life threatening endocarditis. *S. aureus* can carry a vast number of virulence factors but, little is known about their role in pathogenesis. Insight in the possible relation between a specific infection and the expression of certain virulence factors is generally lacking. From previous work, it is known that immunoglobulin subclass switching can be indicative for location and duration of antigen exposure. Here, we attempt to delve into these immune responses to determine differences caused by various interactions between the pathogen and humans.

Methods: To study this for *S. aureus* we developed a dedicated bead-based flow-cytometry technique (xMap, Luminex) that allows us to simultaneously quantify the humoral immune response against up to 40 relevant *S. aureus* virulence factors per test. With this system we studied the anti *S. aureus* immunoglobulin titres (IgG, IgG1, IgG2, IgG3, IgG4, IgA and IgA1) in serum of 10 adult persistent *S. aureus* carriers, 10 adult non-carriers and 10 bacteraemia patients. These groups are compared using the Mann-Whitney U Test in their response against these 40 antigens. Also, (IgNspecific subclass /IgNtotal) ratios are calculated to determine if there are shifts in the responses, from one subclass of antibody to a different subclass. We also performed inhibition Luminex analyses on abscess fluid to directly determine the local expression of *S. aureus* antigens.

Results: The IgG4 responses are measurable against only a small conserved panel of antigens (Chips, Eta, HlgB, LukD, LukE, LukF, Scin, Ssl-1, Ssl-9 and Tsst-1). Interestingly, these antigens are all secreted immunomodulators, with response patterns varying between subjects. Bacteraemia patients have statistically significantly higher IgG4 responses against HlgB and LukF than carriers, and EtB, HlgB, LukS, Ssl-1, Ssl-3 and Tsst-1 than non carriers. Non carriers and carriers only vary in responses against Scin and Tsst-1. To study the potential induction of IgG4 during bacteraemia, longitudinally collected serum samples from the same bacteraemia patients were analysed at diagnosis of bacteraemia, 1 week or 2 weeks post infection. Levels of IgG4 were not altered or changed very little in time, meaning that there is no induction of a humoral response after clinical presentation of bacteraemia.

Conclusions:

1. IgG4 responses were found against only a restricted panel of antigens (Chips. Eta, HlgB, LukD, LukE, LukF, Scin, Ssl-1, Ssl-9 and Tsst-1).
2. The induction of IgG4 responses against these antigens in the bacteraemia group did not change in 2 weeks time after onset of the first positive blood culture.
3. Since IgG4 class switching takes place only after prolonged continuous exposure, this indicates either during colonization and / or during (previous) infections, humans are exposed for a prolonged time to these virulence factors.

P142

Rapid molecular detection of ESBL gene variants with a novel ligation-mediated Real Time PCR

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Introduction: Extended spectrum beta-lactamases (ESBLs) are emerging worldwide. Rapid and adequate ESBL detection is desired for both infection control measures as well as for the choice of correct antimicrobial therapy. In this study we compared a new rapid ligation-mediated Real Time PCR to detect the most prevalent ESBLs TEM, SHV and CTX-M to a phenotypic confirmatory method. Moreover, we evaluated the turn-around-time (TAT) of both methods.

Methods: From June-October 2011 all gram-negative ESBL positive strains from clinical specimens based on VITEK2 results were collected and tested using the phenotypic combination disk test (CDT) and a new ligation-mediated Real Time PCR (LMRT PCR; Checkpoints, Wageningen, the Netherlands). Per patient only the first of each putative ESBL-positive species was included. Disconcordant results were analyzed with the established Check-MDR CT103 assay (Checkpoints) detecting most prevalent ESBLs, pAmpCs and carbapenemases. All isolates, in the period August-October, which were selected for the LMRT PCR and tested in de CDT on the same day (Monday-Thursday starting 11:00hr) were used to compare the TAT.

Results: Of the 195 putative ESBL-producing isolates 106 (54.4%) and 93 (47.7%) were positive using the CDT and LMRT PCR, respectively. Fifteen discrepancies were found of which 14 were phenotypical ESBL positive and genotypical ESBL negative.

In 12 discrepancies, the LMRT PCR results were confirmed with the CT103. In one strain the CT103 could not be interpreted because of lack of amplification spots. The remaining two discrepancies were repeated in the Real Time PCR and detected ESBL positive, comparable to the CDT.

The mean TAT of the CDT and Real Time PCR (24hours 52minutes and 15hours 22minutes respectively) was in favor of the LMRT PCR. In 90,5% of the cases LMRT PCR TAT was shorter than the CDT TAT (varying between 5hr-27min and 24hr-50min).

Of all 63 isolates in which the TAT was evaluated, 31.8% could gain results within the same day using Real Time PCR. In contrast, CDT results only were available the next day because of the overnight incubation step.

Conclusion: The LMRT PCR can easily be introduced in any laboratory having Real Time PCR equipment, thus allowing rapid detection of ESBL. Moreover, this method does not need any post-amplification steps, resulting in a minimal chance for contamination. This is of high importance since cultured isolates are tested for molecular ESBL confirmation.

In conclusion, the LMRT technique provides a new important reduction in TAT for molecular ESBL confirmation with only few laboratory workflow adaptations. As a consequence all ESBL results are available within the same day, making this assay a solid answer to the desire for a rapid and accurate ESBL detection method.

P143

High prevalence of carriage of ESBL-producing *Enterobacteriaceae* in the Dutch community

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Introduction: In a previous study an unexpectedly high prevalence of 10.6% carriage of ESBL-producing *Enterobacteriaceae* (ESBL-E) was found in Dutch patients presenting themselves to their GP with gastrointestinal discomfort or diarrhea in the region of Amsterdam. CTX-M-15 was the most prevalent gene. The aim of the present study was to determine the prevalence of carriage of ESBL-E in the general Dutch community.

Methods: Participants' addresses were obtained from five general practices, affiliated to the Academic General Practice Network, VU University Medical Center, in the region of Amsterdam. Seven thousand participants were approached by postal mail and asked to fill in a short questionnaire and collect a fecal sample or rectal swab. These samples were returned in transport medium (Copan Italia, Brescia, Italy) by mail to our laboratory. Screening for ESBL-E was performed with a selective enrichment broth and inoculation on a selective screening agar, the EbSA ESBL screening agar (Cepheid Benelux, Apeldoorn, the Netherlands). Species identification and antibiotic susceptibility testing were performed with the Vitek-2 system (bioMérieux). ESBL production was confirmed according to the Dutch national guideline.

Results: Of the 7000 persons approached, 1713 participants returned the questionnaire with a fecal specimen. The mean age of patients was 52 years (range: 18-95), 60.5% were females. One hundred and forty four samples (8.4%) yielded ESBL-producing *Enterobacteriaceae*: 131 (91%) Escherichia coli, 11 (7,6%) *Klebsiella pneumoniae*, 1 *Enterobacter cloacae* (0,7%) and 1 *Serratia plymuthica* (0,7%). Of these isolates, 37/144 (25,7%) were resistant to gentamicin, 44/144 (30,6%) to ciprofloxacin, and 88/144 (61,1%) to co-trimoxazole; 11.1% (16/144) were multiresistant (to beta-lactams, gentamicin, ciprofloxacin and co-trimoxazole). Three isolates out of 144 (2,1%) were resistant to nitrofurantoin while 17 (11.8%) were intermediately resistant. All isolates were sensitive to meropenem and imipenem. Results of molecular analyses are pending.

Conclusion:
1. This study confirmed the high prevalence of ESBL-E carriage in the general Dutch community.
2. Among ESBL-E isolates a high percentage of co-resistance to ciprofloxacin, co-trimoxazole and gentamicin was found. Further research is warranted to gain understanding of the epidemiology of this emerging problem in the Netherlands and to find out the origin of this high prevalence in the general Dutch population.

P144

Clinical impact of rapid diagnosis of bloodstream infections: a randomised controlled trial

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Introduction: Bloodstream infections have a high mortality rate. Inadequate empirical therapy is associated with higher mortality. Therefore, broad spectrum antibiotic treatment should be initiated as soon as a bloodstream infection is suspected. But the use of broad spectrum antibiotics is also associated with the emergence of more resistant pathogens.
More rapid methods of identification (ID) and antibiotic susceptibility testing (AST) could result in a reduction in time that an inadequate or too broad spectrum antibiotic is used and thus affect the outcome of the patient, but information on this subject is currently limited.
We therefore conducted a randomised controlled trial to assess the clinical value of more rapid ID and AST in bloodstream infections.
Methods: Adult patients admitted to the Maastricht University Medical Centre were eligible for inclusion if they had a new grown blood culture. Cultures that contained gram-positive rods, anaerobes or a mixed culture were excluded from the study. Written informed consent from patients or their legal representative was required for inclusion. Patients were randomised for either the rapid or the control group. In the control group, routine ID and AST were performed using the BD Phoenix system. In the rapid group, ID was performed using a 16S rDNA based multiprobe PCR. For AST, bacteria from blood cultures were suspended in broth and incubated in the presence of a panel of antibiotics, after which growth or inhibition in each sample was determined by 16S rDNA PCR. Results of the rapid tests were available before 5 PM the same day the blood culture signalled positive. Between October 2009 and May 2011, 223 patients were included: 114 in the rapid group and 109 in the control group.
Results: In the rapid group, 88 patients required antibiotic therapy (26 patients had either a contaminated blood culture or an infected intravascular catheter). Of these, 12 patients (13.6%) switched to a different antibiotic after the results of the rapid test, 5 of which received inadequate empirical antibiotics.
In the control group, 79 patients required antibiotic therapy. After results of the routine method became available, in this group, 29 patients (36.7%) switched to a different antibiotic, as opposed to 20 patients in the rapid group (22.7%, p = 0.049). However, no difference was observed between the groups in the average time that inadequate or too broad spectrum antibiotics were used: 34.4 hours in the rapid group versus 30.6 hours in the control group (95% CI -13.4 - 5.8, p = 0.66). There was no difference in mortality (p = 0.261) or length of stay (p = 0.266).
Conclusion: This study shows that in bloodstream infections rapid ID and AST can result in an earlier antibiotic switch. But no significant reduction could be shown in time that inadequate or too broad spectrum antibiotics were used. Reasons for this could be: the low prevalence of antibiotic resistance in the Netherlands, the relatively large amount of time that is required for a blood culture to signal positive and the low implementation rate of results of rapid ID and AST by medical microbiologists and attending physician.

P145

Mysteries beyond the mycomembrane: identification of genes involved in mycobacterial capsule biogenesis using a random transposon mutagenesis approach

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Introduction: Tuberculosis, caused by Mycobacterium tuberculosis, is the most deadly bacterial disease worldwide and kills over 1.6 million people each year. *M. tuberculosis* possesses a variety of immunomodulatory factors that suppress the host immune response. When the bacillus encounters its target cell, the outermost constituents of its cell envelope are the first to interact. Mycobacteria, including *M. tuberculosis*, are surrounded by a loosely attached capsule that is mainly composed of proteins and polysaccharides. Although the chemical composition is relatively well known, the route of biogenesis and biological function of the capsule are only poorly understood. The aim of this study was to obtain further knowledge on mycobacterial capsule biogenesis by using a random transposon mutagenesis screening approach and select for mutants with an altered capsule production. We focused on alpha-glucan, which is, in *M. tuberculosis*, the major capsular polysaccharide.
Methods: Random transposon mutant banks were created in three representative mycobacterial species: the non-pathogenic species Mycobacterium smegmatis and the two pathogenic species *Mycobacterium marinum* and *M.*

tuberculosis. Obtained transposon mutants were screened for an altered capsule production with an antibody against alpha-glucan in a double filter screening approach. Mutants that displayed an altered alpha-glucan production were selected and re-screened for validation of the phenotype. Transposon insertion sites were determined using ligation-mediated PCR followed by DNA sequencing.

Results: In total, about 40,000 individual colonies were screened from which 140 transposon mutants with an altered capsule production were identified. Ligation-mediated PCR and subsequent sequencing showed that amongst the mutated genes identified were glucose-1-phosphate adenylyltransferase glgC and glycogen branching-enzyme glgB, both of which are known to be involved in capsular alpha-glucan biosynthesis thus confirming the validity of our screening approach. Additional mutations were present in a variety of genes involved in specific cellular processes with a remarkably high number of insertions in genes involved in central carbon metabolism and nutrient acquisition. Furthermore, our screening approach led to the serendipitous identification of a novel class of putative mycobacterial outer-membrane proteins possibly involved in facilitating transport of compounds through the mycobacterial outer membrane.

Conclusion: In this study we have identified a panel of mutants in relevant mycobacterial species with various defects in capsular alpha-glucan production. This result demonstrates that transposon mutant screening is a valid approach to identify genes involved in capsule biogenesis in mycobacteria. Further investigation of these mutants will not only provide more understanding into the process of mycobacterial capsule biogenesis and how this is regulated but will also provide important tools for studying the role of the capsule during *M. tuberculosis* infection.

P146

Nationwide comparison of serological assays for detection of *Borrelia* antibodies in clinically well-defined patients

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Background: Numerous tests for the detection of antibodies against *Borrelia* spp. are commercially available. Manufacturer derived data invariably report a high sensitivity and specificity but comparative studies demonstrate large differences in clinical practice, especially with regard to specificity. Patient organizations in the Netherlands have questioned the sensitivity of serological testing.

Objective: To investigate the sensitivity and specificity of *Borrelia* antibody assays in patients from the Netherlands with clinically well defined manifestations of *Borrelia* infection and/or confirmation with PCR.

Methods: We retrospectively collected data from validation studies for *Borrelia* antibody assays from seven large laboratories in the Netherlands. The total number of samples from which data were available was 810. Samples were selected based on clinical and laboratory parameters. We included samples from patients with erythema migrans, acrodermatitis chronicum atrophicans, neuroborreliosis, arthritis, a large number of cross reactivity controls (CMV, EBV, HIV, leptospirosis, syphilis, *Mycoplasma pneumoniae*, Parvo B19, Reumatoid factor positive) and healthy controls. Data from 10 ELISA's (C6 peptide-Immunetics, Diacheck, Enzygnost, Euroimmun, Liaison, Medac, Mikrogen, Serion, Vidas, Virotech) and five immunoblots (Euroimmun, Mikrogen, RIVM home made, Virablot, Virotech) were analysed. The samples were derived from different laboratories with different screenings assays for *Borrelia* antibodies. 22 patients were *Borrelia* PCR positive.

Results: When IgM and IgG results were combined, sensitivities of the ELISA's varied considerably in patients with short disease duration such as EM. For ACA and arthritis patients, although groups were small, the sensitivity of all tested assays was very high and only minor discrepancies were found between tests. In cross-reactivity controls the fraction of positive samples varied considerably, mainly in IgM alone. Seropositivity rates in healthy controls were low, corresponding with earlier findings concerning seroprevalence in the Netherlands. For the immunoblots, only for the Mikrogen and Virotech blot sufficient data for valid conclusions were available. For these blots, the sensitivity was slightly lower than for the ELISA's, especially in patients with early Lyme disease. For disease manifestations with short duration such as EM and acute neuroborreliosis, up to about 40% of patients had IgM antibodies only, depending on the assay used. With immunoblot the percentage of patients with EM/neuroborreliosis with IgM antibodies 25-30%. In the cross-reactivity controls, the false positive reactions were predominantly IgM antibodies. All 22 PCR positive patients were reactive in at least one assay. Only a few EM patients had a negative result in one or more assays. All PCR positive patients with longer disease duration tested positive for *Borrelia* antibodies.

Conclusions: This nation wide retrospective study demonstrates that for manifestations of *Borrelia* infection with short disease duration, the sensitivity of the assays varies considerably. In patients with long disease duration, sensitivities differ only marginally. In cross-reactivity controls,

there is huge variation in the seropositivity rate, but mainly in IgM. IgM testing only adds in patients with short disease manifestations, in patients with longer duration of illness IgM single positives should interpreted with caution.

P147

Combined evaluation of analytical and clinical performances of five commercially available antibody assays for Epstein-Barr virus

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Introduction: Evaluations of the clinical performances of commercially available Epstein- Barr virus (EBV) immunoglobulin G and M (IgG, IgM) tests are scarce. In this study, we analysed the analytical performances in combination with the clinical predictive values of five commercially available ELISA assays for EBV-viral capsid antigen (VCA) IgG and IgM, -early antigen (EA) IgG, and -nuclear antigen (NA) IgG antibodies: Plexus EBV Multi-Analyte (Focus); EBV-EIA (Biotest), VIDAS (bioMérieux), Liaison (DiaSorin) and Immulite (Siemens Healthcare Diagnostics).
Methods: A panel of 76 acute-phase sera from 37 patients with EBV infection (confirmed by PCR and IgG conversion or titer-increase) and 95 control sera was tested. Control sera consisted of i: sera from patients known to be seronegative (n = 30) and ii: acute-phase sera from patients with HIV (n = 5), parvo-B19 (n = 18), *Cytomegalovirus* (n = 15), Dengue (n = 14) or *Mycoplasma pneumoniae* (n = 13). Equivocal test results were interpreted as negative. Clinical stages of EBV included: no infection, early infection, acute-, latent-, reactive- or undetermined. DNA detection of EBV by PCR was performed using an internally controlled real-time assay.
Results: The analytical performances of the VCA-IgM assays showed sensitivities ranging between 49,2% (Biotest) and 85,7% (Plexus), and specificities ranging between 81.7% (Liaison) and 98.7%. VCA IgM. ROC-AUC values ranged between 0.885 (Biotest) and 0.926 (VIDAS). The EBNA-G sensitivities ranged between 77.9 (Liaison) and 96.0 (Immulite) and the specificities ranged between 70.4 (Immulite) and 100% (Plexus and Biotest). The VCA-IgG and EA-IgG results showed limited variations between the different assays, with the best ROC values for Biotest (0.987) and Biotest (0.989) respectively. The clinical performances varied considerably between the assays tested, with a highest correct clinical diagnosis in 77.0% (VIDAS) and a lowest clinical score of 63,7% (Biotest). Assays that missed acute EBV infections were Immulite and Biotest, whereas latent infections were missed by Plexus and Liaison.
Conclusion: The results of the present study show that the analytical performances varied considerably between the five different EBV antibody assays tested. A combined evaluation of the analytical performance and clinical score provides a better insight into the limitations and possible use of the current EBV antibody assays in clinical practice.

P148

Evolutionary adaptation of *Akkermansia* species within the mammalian host

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Introduction: *Akkermansia muciniphila* is isolated from human intestine and belongs to the *Verrucomicrobia* phylum. The microbe is suggested to be beneficial as it is inversely correlated with IBD, appendicitis, autism and obesity. *A. muciniphila* is abundantly present in the healthy human intestine, varying from 1 to 4% in the colon. It colonizes the mucus layer that covers the colon and can use mucin as sole carbon and nitrogen source.
Akkermansia like sequences are universally distributed among the intestines of mammals. The abundance and distribution of *Akkermansia spp.* along these animals suggests co-evolution with their host and therefore a potential functionality in the GI tract. It is expected that *Akkermansia spp.* have adapted to their host GI tract anatomy and host diet, i.e. to their host phylogeny.
The aim of this study is to make a genomic comparison of new mammal *Akkermansia* isolates to get insights into the co-evolution of mammals with one of their potential symbionts.
Methods: New *Akkermansia* strains were isolated, by using culture media containing mucin as only carbon and nitrogen source. The full-length 16S rRNA gene sequence of each pure culture was determined and compared to the *A. muciniphila* type strain (ATCC BAA-835).
A phylogenetic tree was generated, indicating the position of *Akkermansia muciniphila* among selected full length 16S rRNA clones from mammalian gut samples.
Results: Pure cultures were obtained from 30 different mammalian faecal samples, including 7 human inocula. The 16S rRNA genes from five of the human derived cultures were > 99% identical to the type strain and showed two distinct point mutations in the variable region of the gene.
The tree with *Akkermansia*-related 16S rRNA gene sequences derived from mammalian intestinal samples consists of five distinct clades of which four contain sequences associated to human gut samples. One clade is devoted to primate samples e.g. gorilla, chimpanzee and human derived *Akkermansia* strains, these al share 99-100% similarity to the *A. muciniphila* type strain. The sequence similarity between the type strain of *A. muciniphila* and sequences within the other four clades ranges from 80 to 100%.

Discussion: The 16S rRNA gene sequence similarity between the type strain and other mammalian derived sequences ranges from 80 to 100%. This suggests mammal colonization with different *Akkermansia* species and genera. Future genetic and physiologic characterisations of the new mammalian isolates will verify adaptation of *Akkermansia spp.* to their host. Simultaneously, this characterisation can unravel the functionality of *Akkermansia spp.* in the mammalian gut and their potential symbiotic role.

P149

The use of SpectraCellRA as a bacterial typing tool for gram-negative pathogens

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Introduction: Over the past decades, a significant increase of hospital acquired infections (HAI) caused by gram-negative bacteria has been observed. Acquired antimicrobial resistance in these bacteria is probably the most anticipated problem in hospitals. The four most common gram-negative pathogens associated with HAI are *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter* spp. Effective prevention of HAI requires bacterial typing, since this is a powerful tool for the identification of transmission pathways. In this study we have developed a protocol for the typing of gram-negative bacteria using the SpectraCellRA system

Methods: Several well documented collections containing ESBL-producing bacteria of the above mentioned species 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 (River Diagnostics BV, Rotterdam, the Netherlands) and allowed to dry. Spectroscopic fingerprints were obtained using a SpectraCellRA analyzer (River Diagnostics BV). Similarities between isolates were calculated from the squared Pearson correlation coefficients and displayed in a 2 dimensional (2D) checkerboard plot.

Results: Technological proof-of-principle for efficient, reproducible typing has been obtained for all species mentioned. The discriminatory power for these species matches that of established genotyping techniques. 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 and thus prevent the transmission of microorganisms in an early stage by the implementation of adequate hygienic measures. Due to the concordance to high resolution genetic typing methods, the ease of use, reproducibility and the high throughput, the SpectraCellRA system is a useful typing tool in the battle against HAI.

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Routine typing of *Staphylococcus aureus* and MRSA using SpectraCellRA

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Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) is still an important cause of hospital acquired infections. Efficient infection control and monitoring of this microorganism depends on microbial typing. Previously, a protocol has been developed for the fast and reliable typing of *S. aureus* isolates using the SpectraCellRA system. In this study we have evaluated its benefit in the routine typing and outbreak analysis of this organism.

Methods: Isolates were obtained from routine cultures and contact screenings. For analysis, all isolates were cultured on Trypticase Soy agar plates, biomass was suspended in sterile distilled water, transferred onto a MicroSlide (River Diagnostics BV, Rotterdam, the Netherlands) and allowed to dry. Spectroscopic fingerprints were obtained using a SpectraCellRA analyzer (River Diagnostics BV). Similarities between isolates were calculated from the squared Pearson correlation coefficients and displayed in a 2 dimensional (2D) checkerboard plot.

Results: The SpectraCellRA system is easy to implement in the daily routine of a microbiological laboratory. In possible outbreak situations the system performed as well as established typing techniques, but results could be obtained earlier.

Conclusion: Using the SpectraCellRA system as a routine typing tool for *S. aureus* it is possible to recognize possible outbreak situations early. By building a database of all isolates retrieved on a certain ward or hospital allows the detection of links that might be missed in the current daily routine.

We conclude that SpectraCellRA is an easy-to-use typing tool that can be used for routine typing and therefore provides a good alternative in the battle against MRSA.

P151

Is hydrogen peroxide useful for the degradation of DNA fragments to prevent PCR contamination?

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Quality guidelines for molecular diagnostics are directed towards the prevention of contamination of laboratories and reagents, both by microorganisms and DNA fragments. Despite these guidelines, contaminating incidencts occur and routine checks of the laboratories often show the presence of DNA fragments, which may cause false positive results in highly sensitive PCR assays. Hydrogen peroxide (H_2O_2) has proven to be effective against viruses and bacteria and is used as a room decontamination method both in liquid and vapour form. H_2O_2 is also known to break down the sugar-phosphate backbone of DNA by means of hydroxyl radicals. We tested the efficacy of H_2O_2 in liquid and vapour for its ability to degrade short DNA amplicons. To do so, dilution series (ranging from 10^8 to 10^4 copies per ml) of a 100 bp amplicon were spotted onto Trespa boards and dried. The boards were subsequently subjected to 30% H_2O_2 liquid or decontamination protocol using a hydrogen peroxide vapour (HPV) generator (Bioquell). Following exposure to H_2O_2, the DNA was resuspended and tested using real-time PCR. Exposure to H_2O_2 in liquid or vapour form resulted in approximately a 1-log reduction in the concentration of DNA present, and was not able to prevent a positive PCR reaction compared with unexposed controls even in highly diluted samples. Although theoretically the H_2O_2 decontamination treatment would be expected to be effective, the results of our experiments leave some room for improvement.

P152

Mass spectrometric analysis of the changes in the secretome and wall proteome of *Candida albicans* upon white-opaque switching

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The human fungal pathogen *Candida albicans* causes superficial mucocutaneous infections in healthy individuals as well as life-threatening systemic infections in immuno-compromised patients. Several forms of morphological switching have been described, with yeast-to-hyphae transition being the most important one for virulence. In addition, another form of phenotypic switching, called white-opaque switching, can occur from the typical yeast form (smooth, white colonies; oval cells) to the mating-competent form (flat, opaque colonies; elongated cells with an irregular wall). Importantly, opaque cells become more resistant to neutrophil killing. Cell wall and secreted proteins are important virulence factors that promote successful colonization and invasion of the host tissues as well as immune-evasion. This led us to investigate the wall proteome and the secretome of opaque cells using Liquid Chromatography coupled tandem Mass Spectrometry. We used cultures of the mating-competent strains WO-1, the wildtype-derived hemizygotes SC5314 α and SC5314 a, as well as CAI4+Clp10 homozygotes grown for 18h at 25°C and analyzed their secretome and the wall proteome for both the white and the opaque form. All opaque cultures showed the same changes in the opaque phase, suggesting that the adaptations in the opaque cells were strain-, mating type- and zygosity-independent. Opaque-specific secreted proteins like Cyp5, Op4, Sap1, and Sap99 were detected in secretome samples of opaque cells. Moreover, a significant decrease in the levels of the secreted chitinase Cht1, the phospholipase Plb4.5 and cell wall maintenance proteins Scw11 and Sim1 was observed. On the other hand, only few changes were detected in the wall proteome, with Cht2 being decreased in abundance while Sap9 was detected exclusively in opaque cultures.
In conclusion, despite the significantly altered cell shape, the wall proteome is only slightly affected by white-opaque switching. There are significant opaque-specific changes in the secretome, which are useful opaque marker proteins. These proteins were detected independent of mating type, strain background and homo- or hemizygosity in the mating type locus. Further investigation is required to identify the contribution of these proteins to immuno-evasion and their usefulness in diagnostics.

P153

High resolution typing of livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 using optical mapping enables identification of transmission events

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Introduction: In 2003, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) was identified in the Netherlands and has become the predominant MRSA clade. Typing of LA-MRSA isolates using staphylococcal protein A (*spa*) typing and multiple-locus variable-number of tandem repeat analysis (MLVA) yields insufficient discriminatory power to enable identification of transmission events caused by LA-MRSA. Pulsed-field gel electrophoresis (PFGE) using *Cfr*9I has been shown to provide a much higher discriminatory power, but this technique is labor intensive and has low portability. Recently, a high resolution microbial whole genome analysis named optical mapping was introduced. Like PFGE, this technique creates restriction fragments albeit at a high density (300 fragments) and the fragments are displayed in maps ordered as they occur in the genome. In

this study we assessed the capability of optical mapping to type LA-MRSA and elucidate transmission events.

Methods: LA-MRSA and MRSA isolates (n = 116) were typed by optical mapping, MLVA, *spa*-typing and PFGE. Isolates obtained from outbreaks and from epidemiologically non-related cases were used for the validation of the method. In addition, isolates obtained from 17 veterinarians and their family members were used to study transmission of LA-MRSA. In order to create optical maps, high molecular weight DNA (HMW DNA) was extracted from *S. aureus* using the Argus™ HMW DNA isolation kit and HMW DNA molecules were stretched and immobilized in micro-fluidic channels. The DNA molecules were then digested with restriction enzyme *Afl*II and stained using micro-fluidics. Subsequently fragments were visualized and sized in the Argus™ machine. Using advanced software the fragments were assembled into high resolution restriction maps yielding a set of ordered restriction fragments as they occur in the genome.

Results: The similarity between optical maps created using the same extracted HMW DNA on 5 consecutive days was approximately 99%, demonstrating high reproducibility. Furthermore, the similarity between optical maps of the first and last day sample of isolates that were sub-cultured for 30 days was above 99%. Three well documented outbreaks of both LA-MRSA and MRSA were investigated. Two outbreaks (LA-MRSA and MRSA) could be confirmed. However, the third outbreak of LA-MRSA showed major differences between the optical maps indicating not all isolates belonged to this putative outbreak. Optical maps of LA-MRSA isolates obtained from 17 veterinarians yielded many different types, indicating a high degree of differentiation. In contrast, only 4 different MLVA-types were found among these isolates. Samples obtained from two veterinarians and their family members were further investigated. In one family the optical maps of the veterinarian and his family members were nearly indistinguishable, indicating that transmission had occurred. In contrast, the maps of the other veterinarian and his family members differed considerably, showing colonization had not occurred due to transmission from the veterinarian to his household. Remarkably, PFGE of these isolates showed indistinguishable banding patterns for both families.

Conclusion: Until now PFGE using *Cfr*9I was the best method to differentiate LA-MRSA isolates. However, this new optical mapping technique proved to be a much better and more robust technique to investigate possible transmission events within the LA-MRSA clade.

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