Society for General Microbiology | UK
Society for Medical Microbiology | Norway
Society for Microbiology | Norway
Society for Virology | Norway

Joint Meeting
27–30 September 2005
Radisson SAS Royal Hotel, Bergen | Norway

Fighting infection
Challenges and recent advancements in microbiology

Programme and Abstracts

Contents

Please note: *Abstracts are printed essentially as received from the authors and are not subjected to rigorous editing.*

Meeting at a glance

Tuesday 27 September	Wednesday 28 September	Thursday 29 September	Friday 30 September
	Welcome addresses Introduction 0845	Session 2 *Antimicrobial resistance and new strategies for the development of novel compounds* 0900	Session 3 *Workshop – Microbiology education: dead or alive?* 0900–1300
	Session 1 *New & re-emerging diseases* 0905	Session 3 *Marine microbiology: Fish diseases and their prevention* 1345	
	Poster viewing Trade exhibition 1710–1740	Poster viewing Trade exhibition 1730–1830	
Registration 1600–2000	Civic reception Haakon's Hall 1830–1930		
Welcome reception 2000	Lepra Museum Tour and talk 2000	Conference dinner Schøtstuene, Bryggen 1900 for 1930	

Events

Tuesday 27 September

2000 Welcome reception, Radisson SAS Royal Hotel

Wednesday 28 September

1830–1930 Civic reception, Haakons Hall (kindly provided by the City of Bergen)

2000 Lepra Museum. A tour of the site where Armauer Hansen first identified *M. leprae* in 1873, including a short talk by the curator, Prof. Lorentz Irgens.

The venues are within easy walking distance. Coaches will be provided if it is raining.

Thursday 29 September

1900 for 1930 Conference dinner, Schøtstuene, the historic merchant's hall in the Bryggen. (The cost is not included in the registration fee.) *The hall is a very short walk from the hotel.*

General information

All events will take place in the conference centre of the Radisson SAS Royal Hotel, unless noted otherwise.

Registration

This will take place in the conference centre at the following times:

Tuesday 27 September	1600–2000
Wednesday 28 September and Thursday 29 September	0800 to end of scientific sessions
Friday 30 September	0800–0900

Scientific sessions

These will take place in the lecture theatre of the conference centre.

Name badges

These should be worn at all times to ensure your entry into the scientific sessions and the other events.

Posters

Poster boards are located in the exhibition area of the conference centre. Posters may be mounted on the boards from 0800 on Wednesday, 28 September. Boards will be labelled with the poster number assigned in the abstracts section. Pins will be provided. Only 1 metre by 1 metre is available for each poster.

Presenters should be available by their posters at the times indicated in the scientific programme. Posters should be on display throughout the entire meeting.

Refreshments and lunch

These will be served in the conference centre during the breaks indicated in the scientific programme. Tea, coffee, fruit juice and water will be available with the buffet lunches; alcoholic beverages may be purchased from the hotel bar.

Email

Email can be accessed in the business centre and the bedrooms of the Radisson SAS Royal Hotel. Cards to enable email access may be purchased from the hotel reception.

Parking

Parking is available in the underground garage beneath the hotel. There is a daily fee.

Trade exhibition

This will take place alongside the posters in the conference centre at the following times:

Wednesday 28 September	1000–1740
Thursday 29 September	1030–1830

Scientific programme

Wednesday 28 September 2005

0845	Welcome addresses *Representative of the University of Bergen* *Hugh Pennington, President of the Society for General Microbiology* *Lars Haarr, on behalf of the Norwegian societies*
0900	Introductory comments *Geoffrey Schild, Society for General Microbiology*

Session 1 New and re-emerging infections

Co-chairs ***Del Ala-Aldeen (UK) and Lars Haaheim (Norway)***

0905	Structural studies of influenza HA – clues to the origin of pandemic viruses *John Skehel (MRC National Institute for Medical Research, UK)*
0935	Bacterial diarrhoeal disease – a challenge for development of vaccines *Jan Holmgren (University of Göteborg, Sweden)*
1005	Coffee / Posters set up / Trade exhibition
1025	Tuberculosis: the host–pathogen interaction *Harleen Grewal (University of Bergen, Norway)*
1055	Sexually transmitted infections – global challenges *Arvid Nilsen (University of Bergen, Norway)*
1125	Influenza and vaccines: an update on progress 1. New developments in influenza vaccines *John Wood (National Institute for Biological Standards & Control, UK)* 2. Taming the influenza virus by reverse genetics *Jim Robertson (National Institute for Biological Standards & Control, UK)* 3. Preparedness against pandemic influenza *Lars Haaheim (University of Bergen, Norway)*
1235	Lunch

Co-chairs ***Hugh Pennington (UK) and Geoffrey Schild (UK)***

1330	Vaccines against SARS: where do we stand? *Albert Osterhaus (Erasmus University, Rotterdam, The Netherlands)*
1400	Human transmissible spongiform encephalopathies *James Ironside (University of Edinburgh, UK)*
1430	Natural and experimental scrapie in sheep: studies of pathogenesis and morphology *Cecilie Ersdal (Norwegian School of Veterinary Sciences, Oslo, Norway)*

1500	Coffee / Poster viewing / Trade exhibition
1520	The threat of emerging exotic viruses *Richard Elliot (University of Glasgow, UK)*
1550	Meningococcal infection *Tone Tønjum (University of Oslo, Norway)*
1620	Novel approaches to the development of vaccines: progress on anthrax *Vidadi Yusibov (Fraunhofer, Delaware, USA)*
1650	*Offered paper* Rotavirus vaccines: developments and prospects *Ulrich Desselberger (Health Protection Agency, Cambridge, UK)*
1710	Poster viewing – authors present / Trade exhibition
1740	End of session

Thursday 29 September 2005 (a.m.)

Session 2	Antimicrobial resistance and new strategies for the development of novel compounds
Co-chairs	*David Hopwood (UK) and Harald Wiker (Norway)*
0900	Molecular genetics in the discovery of new antibiotics *David Hopwood (John Innes Centre, Norwich, UK)*
0930	The epidemiology and genetics of antibiotic resistance *Johanna E. Sollid (University of Tromsø, Norway)*
1000	Antiviral drugs: old and new *Eric De Clercq (Catholic University, Leuven, Belgium)*
1030	Coffee / Poster viewing / Trade exhibition
1045	Public health strategies for fighting antimicrobial resistance *Gunnar Skov Simonsen (University of Tromsø, Norway)*
1115	Influenza viruses resistant to neuraminidase (oseltamivir) are biologically compromised *John Oxford (Retroscreen Virology Ltd, London, UK)*
1145	*Offered paper* The effect of antimicrobials on multi-drug-resistant bacterial biofilms ***K. Smith**, C.G. Gemmell, J.G. Anderson & I.S. Hunter (University of Strathclyde and University of Glasgow, UK)*
1200	*Offered paper* Epidemiological analysis of MRSA in two counties in Western Norway ***L. Kindingstad**, S. Fylkesnes, S. Harthug, H. Mylvaganam & H.G. Wiker (Haukeland University Hospital, Bergen, Norway)*
1215	*Offered paper* Antibiotic-producing actinomycete bacteria from the Trondheim fjord ***H. Bredholt**, E. Fjærvik, S. Hakvåg, H. Jørgensen, K.D. Josefsen. H. Sletta, G. Johnsen, T.E. Ellingsen & S.B. Zotchev (NTNU, Trondheim, Norway)*
1230	Award winner's presentation
1245	Lunch

Thursday 29 September 2005 (p.m.)

Session 3	Marine microbiology: fish diseases and their prevention
Co-chairs	*Brian Austin (UK) and Curt Endresen (Norway)*
1345	Emerging fish diseases *Brian Austin (Heriot-Watt University, Edinburgh, UK)*
1415	Fish vaccines – progress and perspectives *Odd Magne Rødseth (Aqua Gen AS, Trondheim, Norway)*
1445	Infectious salmon anaemia virus – an 'influenza' virus of fish *Bjørn Krossøy (Intervet Norbio AS, Bergen, Norway)*
1515	Coffee / Poster viewing / Trade exhibition
1600	Glycoproteins of infectious salmon anaemia virus *Vidar Aspehaug (University of Bergen, Norway)*
1630	Perspectives on the design of DNA fish vaccines *Colin Howard (The Royal Veterinary College, London, UK)*
1700	*Offered paper* The fish larval intestine – a battleground for pathogens and probionts? *Øivind Bergh (Institute of Marine Research, Bergen, Norway)*
1715	*Offered paper* Bactericidal killing mechanisms in the lesser spotted catshark (*Scyliorhinus canicula*) ***A.C. Carrington**, A. Goostrey, J. Reddick & C.J. Secombes (University of Aberdeen, UK)*
1730	Poster viewing – authors present / Trade exhibition
1830	End of session

Friday 30 September 2005

Session 4	Workshop – Microbiology education: dead or alive?
Co-chairs	*Susan Assinder (UK), Joanna Verran (UK) and Hans Utkilen (Norway)*
0900	Introduction by the chairs At the beginning of each session there will be short presentations followed by discussion
0910	Is any news good news? The public face of microbiology *Hugh Pennington (University of Aberdeen, UK)* *Tone Tønjum (University of Oslo, Norway)*
0950	Making the best of a bad job – microbiology in schools (ages 5–18) *Susan Assinder (University of Wales, Bangor, UK)* *Nils Olav Sjøberg (Oslo University College, Norway)*
1030	Coffee
1100	The threat of emerging degrees – undergraduate education *Joanna Verran (Manchester Metropolitan University, UK)* *Gunnar Bratbak (University of Bergen, Norway)*
1140	Building on crumbling foundations – careers in microbiology *Hans Utkilen (The Norwegian Institute of Public Health, Oslo, Norway)* *Colin Howard (The Royal Veterinary College, London, UK)*
1220	Forum – the way forward
1300	Lunch sponsored by the Society for General Microbiology

Speakers – Abstracts

Session 1 New and re-emerging infections

Structural studies of influenza HA – clues to the origin of pandemic viruses

John Skehel
MRC National Institute for Medical Research, UK

Abstract not received

Bacterial diarrhoeal disease – a challenge for development of vaccines

Jan Holmgren
University of Göteborg, Sweden

Abstract not received

The host–pathogen interaction in TB with a special focus on the infecting strain

Harleen Grewal
The Gade Institute, Section of Microbiology and Immunology, University of Bergen, Norway

Mycobacterium tuberculosis (MTb) is an intracellular pathogen that survives within macrophages; however, only a small percentage of infected individuals develop the disease. In the majority of cases, the infected individual mounts an effective immune response and successfully controls the infection. The dynamic interaction between pathogen and host may lead to protection, disease or long-term persistent infection. The initial interaction between the macrophage and the mycobacterium is thought to play a key role in determining the outcome of infection. Mycobacterial proteins and lipids are important for virulence and persistence. As a species *M. tuberculosis* exhibits very little genomic diversity and most of the detected variability till date, has been associated with transposable elements. However, recent studies show that polymorphisms among MTb strains can be more extensive than previously foreseen. Genetic variation may play an important role in pathogenesis and immunity. Mycobacterial proteins and lipids important for virulence and persistence as well as an overview on strain variability and consequences for the host will be discussed.

Sexually transmitted infections – global challenges

Arvid Nilsen
Dept of Dermatovenerology, Institute of Medicine, University of Bergen, Norway

Although sexually transmitted infections (STIs) are underreported and underrecognized, they are a major source of morbidity, mortality, and represent a major socioeconomic cost in developing and developed nations.

Genital chlamydia infections are very common globally, whereas – with some important exceptions – most other curable STIs have decreased in the western world. The development of nucleic-acid amplification tests has improved diagnostic procedures. Unfortunately, many of these tests are unavailable for populations where they are most needed. Single-dose azithromycin has improved compliance in the treatment of chlamydia infections. On the other hand, quinolones are rapidly becoming ineffective for gonorrhoea. The present data regarding

STIs facilitating the transmission (infectivity) and/or acquisition (susceptibility) of human immunodeficiency virus (HIV) will be summarized and discussed, in particular the association between herpes simplex virus type 2 (HSV-2) and HIV. The presentation will mainly be based on the present literature, but some additional original data from STI cohorts in Norway and Tanzania will be presented. In Tanzanian STI patients a dramatic increase in HSV-2 seroprevalence during the 90s has been documented, and 80% of GUD cases are caused by HSV-2. In an area with high HIV prevalence, this calls for some kind of intervention.

New developments in influenza vaccines

John Wood

NIBSC, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, UK (e jwood@nibsc.ac.uk)

Conventional inactivated influenza vaccines produced in eggs have been virtually unchanged for 30 years and have been very successful in reducing the impact of influenza in susceptible populations. However there is some room for improvement, particularly in the methods used for vaccine production and in the capacity to broaden the spectrum of immunity induced by vaccines. There is also a need to improve the capacity to produce vaccines against pandemic influenza.

New technologies for vaccine production include reverse genetics, mammalian cell culture and production of recombinant proteins. New strategies for vaccination include the use of live attenuated vaccines, adjuvants and delivery systems for parenteral and mucosal use of inactivated vaccines, and use of conserved virus proteins and DNA vaccines.

Taming the influenza virus by reverse genetics

Jim Robertson

NIBSC, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, UK (e jrobertson@nibsc.ac.uk)

The reverse genetics technique has opened up the horizon for novel research into influenza virus and influenza vaccines. The technique is being used to investigate novel types of influenza vaccine and can be applied now to generate candidate vaccine strains on a more efficient and rational basis.

The technology was crucial in deriving a candidate vaccine strain for the recent outbreak of human H5 avian influenza in SE Asia. The wild type H5N1 viruses are too virulent to use directly for vaccine manufacture. However, our knowledge of the genetics underlying the high pathogenicity of these strains allows us to engineer out the pathogenic trait using the reverse genetics approach. The rapidity and robustness of the method allowed us to generate a non-pathogenic vaccine reference strain against A/Viet Nam/1194/04 within three weeks of receipt of the wild type pathogenic virus. This was also achieved using a quality system to ensure the suitability of the strain for use in vaccine manufacture and this virus is now being used worldwide for the manufacture and clinical trial of a human H5 vaccine.

Preparedness against pandemic influenza

Lars R. Haaheim

Influenza Centre, The Gade Institute, University of Bergen, N-5021 Bergen, Norway

It is 37 years since the world's last influenza pandemic. Vaccination is the optimal prophylactic measure, but current vaccine production technology will deliver too few doses too late. A pandemic vaccine will be a scarce global commodity and international sale and distribution may be severely restricted or even banned by political interventions. Similarly, the global production of oseltamivir is grossly inadequate and also too expensive for most nations.

With more than 100 human cases of H5N1 avian influenza reported in SE Asia since late 2003, with about 50 human deaths and 140 million dead or destroyed poultry, the situation is alarming. The virus has proven to be genetically unstable and has branched into diverse genetic clusters. Some cases of man-to-man spread have been identified.

With no vaccine or drug intervention, with an attack-rate between 20–40%, with a hospitalization rate of 3–10% for those infected, with a lethality rate of 1–5%, one may calculate that the world may face 1,300–2,600 million infected, 40–260 million in need of hospitalization and 13–130 million deaths.

Recalling the impact of the 8,000 SARS cases in 2003 on world trade, an influenza pandemic could be dramatic. Today's globalized industry operates with very limited stocks of spare parts and raw materials; outputs depend on an uninterrupted international flow of goods. A slump in world trade could obstruct any *ad hoc* emergency procurement of essentials ordered by governments trying to cope with the crisis.

In the aftermath politicians will be asked why they did not act more decisively upon the many warnings. We may have a unique window of opportunity to join forces with the vaccine and drug manufacturers, stockpile essential goods, and fine-tune national pandemic preparedness plans. It is a governmental responsibility – and not that of the WHO and the pharmaceutical industry – to protect their nationals. Also, when the poor nations of the world realize that equitable quantities of the scarce supplies of vaccines, drugs and medical essentials will not come their way, the post pandemic international scene could be one of deep distrust for many years.

Vaccines against SARS: where do we stand?

Albert Osterhaus
Erasmus University Rotterdam, The Netherlands

After the identification of SARS-CoV as the aetiologic agent of SARS and the rapid development of macaque, ferret and rodent models, studies concerning the pathogenesis and the development of intervention strategies were vigorously pursued. Pegylated interferon-α proved to be the first clinically available drug that was effective in preventing and post-exposure treatment of SARS in the macaque model. Subsequently passive transfer of neutralizing polyclonal and monoclonal antibodies was shown to significantly reduce the SARS-CoV load in the respective animal models, when administered preventively. Several research groups started the development of candidate SARS vaccines, using different classical and state-of-the-art technologies. It was realized that besides efficacy, safety of the candidate vaccines should be given special attention, since it has been shown that vaccination of cats with candidate feline coronavirus vaccines predisposed them for antibody mediated enhanced susceptibility upon challenge. In addition, adjuvanted inactivated whole virus vaccines should be given special attention in this respect, since the use of candidate measles and RSV vaccines of this type have predisposed children for more serious disease upon infection in the past. Since macaque models have been developed to study this phenomenon with these two candidate vaccines, it was decided to specifically demonstrate the absence of this phenomenon with inactivated candidate SARS vaccines in macaques. Besides inactivated whole virus vaccines, recombinant-, (MVA-; adeno-), vectored and plasmid DNA vaccines have now been studied in animal models for SARS. In general, these are aiming at the induction of a specific immune response against the S protein of the virus. Most of the candidate vaccines studied so far proved to be protective in the animal models used, with S protein specific virus neutralizing antibodies as the most likely correlate of protection. No antibody-mediated enhancement has been observed, but there are

indications that some of the vaccines tested may predispose for the development of immune pathology upon challenge. The first phase I clinical study has now been carried out in the Peoples Republic of China.

Collectively, the data show that the collaborative activities by several research groups have not only resulted in the rapid control of the SARS outbreak, but also to the development of preventive and therapeutic strategies and will soon lead to the availability of effective and safe vaccines against SARS.

Human transmissible spongiform encephalopathies

James W. Ironside
National CJD Surveillance Unit, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK

Human transmissible spongiform encephalopathies or prion diseases are fatal neurodegenerative conditions that occur in sporadic, familial and acquired (transmitted) forms. All are associated with the accumulation of an abnormal isoform of the prion protein (PrP) in the brain, which is very closely associated with infectivity and may represent the entire infectious agent. The commonest of these is the sporadic form of Creutzfeldt–Jakob disease (CJD), which is a worldwide disorder occurring around 1–2 cases per million population per annum. There is a naturally occurring polymorphism at codon 129 in the PrP gene and homozygosity at this locus is a predisposing factor for sporadic CJD. Variant CJD, which was first described in 1996 and is associated with human exposure to the bovine spongiform encephalopathy (BSE) agent. Infectivity in variant CJD is detectable outside the CNS and accumulates in lymphoid tissues. Two cases of variant CJD infection have been identified in the UK in recipients of blood transfusions from asymptomatic donors who subsequently died from variant CJD. It is extremely difficult to predict the future numbers of variant CJD cases in the UK and elsewhere, so continuing clinical and pathological surveillance is essential for accurate diagnosis, epidemiological studies and health care planning.

Natural and experimental scrapie in sheep: studies of pathogenesis and morphology

Cecilie Ersdal
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Scrapie in sheep is the prototype of a group of fatal central nervous system (CNS) diseases called transmissible spongiform encephalopathies. These diseases are characterized by vacuolation of neuropil and neurons and accumulation of the disease specific prion protein (PrP^{Sc}), an abnormal form of a host-encoded prion protein. In sheep, susceptibility to scrapie is dependent on polymorphisms in the PrP gene. Valine (V) at codon 136 and glutamine (Q) at codon 171 are scrapie promoting.

Orally inoculated and naturally infected sheep with scrapie were examined for PrP^{Sc} using immunohistochemistry. In the inoculated group, $V_{136}R_{154}Q_{171}$/VRQ sheep generally accumulated more PrP^{Sc} in peripheral tissues, as compared with VRQ/ARQ animals at corresponding time points. All inoculated VRQ/VRQ sheep, but only one of eight inoculated VRQ/ARQ animals, were PrP^{Sc}-positive in the CNS. Tissue and cellular localization of PrP^{Sc} suggested lymphatic, periaxonal and hematogenous dissemination. Satellite cells were early targets in the peripheral nervous system. One subclinically affected sheep showed widespread PrP^{Sc} accumulation in the CNS, while three sheep had early clinical signs without detectable PrP^{Sc} in the CNS.

The dorsal motor nucleus of vagus and the olivary nuclei of scrapie-affected sheep were examined by electron microscope. The nature and magnitude of sub-cellular changes differed between the two areas, and neurons in the two neuroanatomic sites therefore appear to process and respond to the presence of PrP^{Sc} differently.

References Ersdal, C., Simmons, M.M., Goodsir, C., Martin, S. & Jeffrey, M. (2003). Sub-cellular pathology of scrapie: coated pits are increased in PrP codon 136 alanine homozygous scrapie-affected sheep. *Acta Neuropathol* 106, 17–28; Ersdal, C., Simmons, M.M., González, L., Goodsir, C., Martin, S. & Jeffrey, M. (2004). Relationships between ultrastructural scrapie pathology and patterns of abnormal prion protein accumulation. *Acta Neuropathol* 107, 428–438; Ersdal, C., Ulvund, M.J., Espenes, A., Benestad, S.L., Sarradin, P. & Landsverk, T. (2005). Mapping PrP^{Sc} propagation in experimental and natural scrapie in sheep with different PrP genotypes. *Vet Pathol* 42, 250–274.

The threat of emerging exotic viruses

Richard M. Elliott
Division of Virology, Institute of Biomedical and Life Sciences, University of Glasgow
(Present address: Biomolecular Sciences Building, School of Biology, University of St. Andrews, The North Haugh, St. Andrews KY16 9ST, UK)

Over the past 30 or 40 years, we have seen the emergence of a multitude of new viral diseases. Many of these can be attributed to man's impact on our planet, such the destruction of natural habitats to development, urbanization, ease of global travel, intensive farming methods and international trade in exotic animals and their products. In this talk some examples of emerged exotic viruses will be reviewed and the continuing threat will discussed.

Meningococcal genome instability and pathogenesis

Stephan A. Frye, Tonje Davidsen, Reza Assalkhou, Seetha Balasingham, O. Herman Ambur, Hanne K. Tuven, Afsaneh V. Benam, Katrina L. Tibbals, Håvard Homberset & Tone Tønjum
Centre for Molecular Biology and Neuroscience and Institute of Microbiology, Rikshospitalet, University of Oslo, N-0027 Oslo, Norway

Neisseria meningitidis or the meningococcus is a leading cause of bacterial meningitis and septicaemia worldwide, also in Norway. Infections caused by meningococci are associated with significant morbidity and mortality for their exclusive human host, affecting mainly small children and teenagers. Type IV pili, which are filamentous structures emanating from the bacterial surface, enable the meningococci to adhere to and colonize mucosal surfaces, as a prerequisite for infection. They are also required for natural competence for transformation. Most evidence suggests that transformation provides the predominant means for exchanges which alter genomic DNA in *N. meningitidis*.

A whole machinery of components is required for pilus biogenesis, assembly, extrusion and retraction. Secretins are a large family of bacterial proteins associated with membrane translocation of macromolecular complexes. A subset of this family, termed PilQ proteins, are required for type IV pilus biogenesis in meningococci. Meningococcal PilQ is unique among secretins because of its abundance in the outer membrane and its N-terminally located polymorphic region containing repetitive elements. Our data indicate that the PilQ complex is the pore through which its substrate, the moving pilus fibre (polymerized PilE), is directed to the bacterial surface. Meningococcal PilQ can induce bactericidal antibodies, making it relevant as a meningococcal vaccine candidate. The lipoprotein PilP is thought to be important for PilQ complex stabilization.

New genetic techniques have allowed us to construct defined mutants to characterize functional domains and detail the dynamics of PilQ complex interaction with other components such as outer membrane proteins and nucleic acid during pilus biogenesis, pilus retraction and DNA uptake in transformation.

Novel approaches to the development of vaccines: progress on anthrax

Vidadi Yusibov, Konstantin Musiychuk, Vadim Mett, Natalia Ugulava, Marina Skarjinskaia & Shailaja Rabindran

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We report here a novel approach to engineer and produce candidate vaccines against a variety of pathogens, including anthrax and flu. This approach has several components: i) a carrier molecule that is based on a thermo-stable protein, β-1,3-1,4-glucanase (LicKM) from *Clostridium thermocellum* that maintains thermostability when fused to large targets, ii) plant virus-based vectors that can be rapidly engineered or modified, and provide high levels of target proteins, and iii) clonal root cultures generated from a single virus-infected plant cell. These cultures are not genetically modified, and the virus-based expression vector is maintained exclusively in the cytoplasm as self-replicating RNA molecules. LicKM facilitates downstream processing and enhances pathogen-specific immune responses. The plant virus/clonal root culture system provides a means for controlled and contained production.

We have engineered, separately, portions of PA (145 aa fragment; Domain 4) and LF (N-terminal 220 aa fragment) as internal fusions in the surface loop of LicKM. Each recombinant protein was purified and evaluated in a mouse model for protective efficacy. Mice immunized (parenterally and intranasally) with three doses of each recombinant antigen or a mixture of the two antigens, mounted strong neutralizing antibody responses and were protected when challenged with lethal dose of anthrax toxin.

Rotavirus vaccines: developments and prospects

Ulrich Desselberger

Cambridge, UK (e udesselberger@btinternet.com)

Rotaviruses (RVs) cause acute gastroenteritis in infants and young children and are endemic worldwide. The burden of disease is high, and in countries under development the mortality per annum amounts to more than half a million children under the age of 2 years. Many viruses of different serotypes cocirculate, serotype G1 to G4 strains being the most commonly found in countries of temperate climate, but other serotypes being detected at high incidence in tropical countries. Repeated natural infection elicits a broad protection against RV disease, the presence and concentration of secreted IgA copro-antibodies being the best correlate of protection.

Since RVs were recognized as a cause of severe human disease, attempts were made to develop a vaccine. After many years of research and successful phase III clinical trials, a live-attenuated, tetravalent (TV), rhesus rotavirus (RRV)-based, human reassortant vaccine (*Rotashield*^{RTM}, Wyeth) was licensed for universal use in the USA in August 1998 and applied to 1.5 million children during the following 10 months. By June 1999, a Vaccine Adverse Event Reporting Scheme (VAERS) had discovered 15 cases of gut intussusception (IS) in vaccinated children, and the vaccination programme was temporarily suspended in July 1999. In October 1999, CDC and the CID of the AAP withdrew their recommendation for the vaccine, and its production was stopped.

In the following years a sharp controversy about the size of the risk of vaccine-associated (va) IS arose as well prolonged discussions about the possible pathogenesis of va IS events. On the other hand, new vaccine candidates were developed. The two vaccine candidates furthest progressed towards licensure are both live attenuated vaccines.

*RotaTeq*RTM (Merck) is a pentavalent, bovine rotavirus-based, human reassortant vaccine eliciting antibodies against serotypes G1 to G4 and P1A[8]. Due to the possible va risk of IS, phase III trials had to be very large safety trials. To test RotaTeq, a total of more than 65,000 children were investigated in a double blind, placebo-controlled study, and no va risk of IS was found.

*Rotarix*RTM (GlaxoSmithKline) is derived from a human rotavirus isolate and is monotypic, eliciting antibodies against serotype G1P1A[8]. In a double blind, placebo-controlled phase III clinical trial involving more than 65,000 children, the vaccine was found not to be associated with IS.

License application for both vaccine candidates are in the process of being submitted in many countries, Rotarix has already been licensed in Mexico. Other vaccine candidates are killed viruses, virus-like particles (VLPs, produced from baculovirus recombinants), DNA-based vaccines, and possibly 'edible vaccines' (viral antigens expressed in plants). The main assurances needed for vaccine licensure, possible barriers to vaccine implementation, and multinational initiatives to introduce licensed vaccines will be reviewed.

Session 2 Antimicrobial resistance and new strategies for the development of novel compounds

Molecular genetics in the discovery of new antibiotics

David A. Hopwood
John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

During the Golden Age of natural product discovery, in the 1950s and 1960s, dozens of antibiotics were developed to treat bacterial and fungal infections, mostly natural products produced by the soil-living, filamentous actinomycetes (for example tetracycline, erythromycin, kanamycin, rifamycin, candicidin...). After the Golden Age, the search for antimicrobial agents became increasingly fruitless. New antibiotics are again in urgent need, especially to combat the rise of multi-drug-resistant *Staphylococcus*, *Enterococcus* and *Mycobacterium* infections, as well as those caused by Gram-negative pathogens. One approach to the challenge is to harness the power of *Streptomyces* genetics to develop 'unnatural natural products' by genetic engineering, especially using the large and supremely important chemical family of polyketides, whose synthesis on giant protein templates programmed to introduce variation into their products lends itself to combinatorial biosynthesis. The scope for such an approach is being widened as large numbers of novel gene clusters are found through large-scale genome sequencing. Often such genes are not expressed under typical culture conditions in the laboratory, but are available through genomics, and the challenge is to find generic ways to switch them on.

The epidemiology and genetics of antibiotic resistance

Johanna U. Ericson Sollid
Dept for Microbiology and Virology, Institute of Medical Biology, University of Tromsø, Tromsø, Norway

Therapy failure due to antimicrobial resistance is a large and increasing problem in both human and veterinary medicine, well documented by epidemiological studies of incidence and

possible outbreaks of infections caused by antibiotic-resistant bacteria. Molecular techniques give us the opportunity to study the incidence and transmission of antimicrobial resistance genes in bacterial populations. Antimicrobial resistance can either spread clonally by bacterial cell division or by horizontal transfer of genetic elements between bacterial cells. To study the distribution and determinants of antimicrobial resistance sophisticated genetic tools are required to differ between bacterial strains within a species as well as specific resistance determinants.

Examples of molecular genetic analyses will be presented to illustrate how antimicrobial resistance determinants can spread in bacterial populations, not only between strains but also between species.

Antiviral drugs: old and new

Erik De Clercq

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The current armamentarium of antiviral drugs encompasses the nucleoside reverse transcriptase (RT) inhibitors (NRTIs) zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir and emtricitabine, the nucleotide RT inhibitor (NtRTI) tenofovir disoproxil fumarate, the non-nucleoside RT inhibitors (NNRTIs) nevirapine, delavirdine and efavirenz, the protease inhibitors (PIs) saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir and fosamprenavir, and the fusion inhibitor (FI) enfuvirtide, for the treatment of human immunodeficiency virus (HIV) infections; lamivudine and adefovir dipivoxil, for the treatment of hepatitis B virus (HBV) infections; acyclovir, valaciclovir, penciclovir, famciclovir, idoxuridine, trifluridine and brivudin for the treatment of herpes simplex virus (HSV) and/or varicella-zoster virus (VZV) infections; ganciclovir, valganciclovir, foscarnet, cidofovir and fomivirsen for the treatment of cytomegalovirus (CMV) infections; amantadine, rimantadine, zanamivir and oseltamivir for the treatment of influenza virus infections; ribavirin for the treatment of respiratory syncytial virus (RSV) infections; and the combination of (pegylated) interferon-α with ribavirin for the treatment of hepatitis C virus (HCV) infections. In recent years, novel selective antiviral agents have been developed with promising activity against any of the aforementioned viruses, particularly HIV, HBV, HCV, HSV and VZV, but also other viruses (such as papilloma, adeno, pox and hemorrhagic fever viruses) that have so far not proven amenable to antiviral chemotherapy.

Public health strategies for fighting antimicrobial resistance

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Antimicrobial drug resistance (AMR) jeopardizes the effective treatment of bacterial, viral, fungal and parasitic infections worldwide and has thus become a global public health problem. The emergence and spread of AMR are the result of the selective pressure exerted by use of antimicrobial agents and the transmission of resistant micro-organisms both in hospitals and in the community. Overuse and misuse of antimicrobial agents result from poor prescribing behaviour, uninformed patient demand and lack of adherence to the treatment regimen prescribed. Low-quality drug formulations, inadequate dosage regimens and insufficient duration of therapy are also important contributors to AMR. The aggregation of highly susceptible patients, the intensive and prolonged use of antibiotics and the lack of implementation of standard practices for infection control are often responsible for the emergence, selection and spread of multi-resistant pathogens in hospitals and other health-care settings. Inappropriate use of antimicrobials also extends to the veterinary and

agricultural fields. Large volumes of antibiotics are administered to food-producing animals for prophylaxis, treatment and growth promotion purposes. About 80% of these administrations have been reported to be unnecessary and this practice may affect human health both because of the presence of drug residues in foods and the selection of resistant bacteria in animals.

In 2001, WHO released the *Global Strategy for Containment of Antimicrobial Resistance*. The Global Strategy includes 14 priority interventions and 67 recommendations in the areas of advocacy, education, management and regulation of drug use. However, the effective management of AMR in a public health context is challenging because AMR is a group of problems involving diverse pathogens transmitted in unique ways that cause a wide range of clinical syndromes. As a consequence there is no single intervention likely to be completely effective in preventing and containing AMR and it has proved difficult to develop integrated public health programmes for this purpose. Furthermore, difficulties in documenting both the impact of AMR and the results of isolated or grouped interventions to control it have hampered the identification of simple strategies and targets for containment programmes. In addition, powerful market forces influence the development, distribution and use of antimicrobial drugs in human and veterinary medicine. These forces have the potential to stimulate the development and distribution of new drugs, but may at the same time promote overuse and misuse of antimicrobials and thus act counter to AMR containment programmes. In recent years, the lack of economic incentives combined with increasing development costs has also led to a decreased interest in the development of new antimicrobial drugs.

Reformulations of existing programmes for AMR containment as well as expansion of the knowledge base about effective interventions at institutional and societal levels are urgently needed. Considerable investment of intellectual and financial resources will be required to achieve these goals.

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Influenza viruses resistant to neuraminidase (oseltamivir) are biologically compromised

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Influenza A and B viruses resistant to the antiviral effect of the neuraminidase inhibitors Relenza and Tamiflu have been detected by *in vitro* passage in the laboratory and from clinical isolates from persons being treated with the drug. Typical mutations are at 119, 272, 274, and more recently at 294. Since a biological balance of necessity is achieved between the receptor binding function of the virus HA protein and the receptor destroying action of the NA protein, mutations in HA can compensate for NA reduced, or blocked, activity. A very relevant property of the NA drug-resistant mutants investigated worldwide, to date, is reduced replication *in vitro* and reduced transmission and pathogenicity *in vivo*. Our data will concern the ferret model of influenza, acknowledged to be the most akin to the human infection. This has been highly refined since the first description of the

model in 1933. We demonstrate both reduced transmission (infectiousness) and pathogenicity in the model system and describe the context of the most recent EU network of viral drug resistance, Virgil.

The effect of antimicrobials on multi-drug-resistant bacterial biofilms

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In recent years there has been an alarming increase in hospital-acquired infections caused by multi-drug-resistant bacteria. Bacterial biofilms can form in the hospital environment on almost any surface. A biofilm comprises a functional consortium of cells enveloped within a matrix of extracellular polysaccharides. The process results in anchoring of the bacteria in a nutritionally-advantageous environment, which protects them from environmental pressures, while regulating population growth. Resistance to antimicrobials is a general feature of all biofilms. Reports have shown that multi-drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), have been isolated from disinfected locations in hospitals, such as catheters and disinfectant soap dispensers. Bacterial biofilms on hospital surfaces may be an important source of multi-drug-resistant bacteria that cause persistent infection. The minimum bactericidal concentration (MBC) of a range of biocides commonly used in hospitals, including chlorhexidine, triclosan and benzalkonium chloride, was determined for a number of multi-drug-resistant organisms grown in biofilms. The effects of these biocides on biofilms grown on different hospital surfaces were also determined. This study has produced clinically-relevant information that is vitally important in guiding improved disinfection practices within hospitals.

Epidemiological analysis of MRSA in two counties in Western Norway

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The isolates of MRSA collected in Western Norway in the period 1992–2004 have been analysed by pulsed-field gel electrophoresis (PFGE). Altogether 197 isolates, from both clinical infections and colonized patients, were analysed by PFGE using the HARMONY protocol. The BioNumerics software (Applied Maths, Belgium) was used for profile interpretation. Clonal relationships based on the PFGE profiles were interpreted according to the criteria of Tenover *et al*. PFGE and profile interpretation revealed that the 197 MRSA isolates could be divided into 38 different PFGE profiles, with 14 of the profiles consisting of more than one isolate. Several of the profiles consisted of subgroups of closely and possibly related PFGE-patterns, as given in the criteria for clonally relationship1. Six major clones comprised 74% of the isolates. The increase in number of new clones indicates that MRSA is an increasing problem in our health region. Continuous surveillance of the clonal relationship between MRSA isolates is important when monitoring the situation. Both PFGE and MLST are well suited for epidemiological analysis, and give valuable information in MRSA surveillance. The methods are however labour-intensive, and the establishment of faster methods would be desirable.

Antibiotic-producing actinomycete bacteria from the Trondheim fjord

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In view of the rapid spread of antibiotic-resistant pathogens causing life-threatening infections, the demand for new antibiotics continues to grow. Although considerable progress is being made toward chemical synthesis of new antibiotics, and generation of novel anti-infectives through genetic engineering, nature still remains the richest source for new antibiotics. Bacteria belonging to the family *Actinomycetaceae* are well known for their ability to produce secondary metabolites, many of which are active against pathogenic micro-organisms.

We have decided to focus on isolation of potential antibiotic-producing actinomycetes from the marine environment in the Trondheim fjord, since it has been demonstrated that some actinomycetes isolated from the sea produce novel and structurally unusual antibiotics. We are pursuing two parallel strategies, isolating actinomycete bacteria from the marine sediments and the upper layer of a water surface (neuston layer). So far, over 5,000 actinomycete colonies have been isolated, and the methods for efficient screening of their antimicrobial activities using the robot lab have been established. Primary screen utilizing Gram-positive bacteria and non-filamentous fungi as test organisms have shown that over 70% of our isolates exhibit antagonistic activity. We are also working on the development of methods for rapid identification of actinomycetes using PCR techniques that should enable us to assemble an annotated library of antibiotic-producing actinomycetes isolated from the Trondheim fjord. The strategies for isolation and identification of novel antibiotics using HPLC, LC-MS/MS and TOF equipment are being developed. The latest data on this project's progress and upcoming challenges will be presented.

Session 3 Marine microbiology: fish diseases and their prevention

Emerging fish diseases

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With the rapid expansion of aquaculture, worldwide, there has been a steady increase in the number of new and/or emerging diseases. In part, this increase may reflect better diagnostic methods, improvements in microbial taxonomy, and the introduction of animal species into new geographical areas. Other reasons focus on changes in the environment, alterations to the health status of the host (perhaps reflecting external stressors such as pollution) and natural selection by which an environmental change selects for a 'new' pathogen. An example could reflect persistent antibiotic usage selecting for antibiotic-resistant pathogens. The increasing number of Gram-positive cocci (e.g. *Lactococcus garvieae* and *Streptococcus iniae*) associated with disease reflects in part improved taxonomic-identification-diagnostic procedures. However, the sudden emergence of *Piscirickettsia salmonis* in Chilean salmon farming may have reflected the spread of an organism from an indigenous to an introduced fish species. Overall, there has been a steady increase in the number of fish/shellfish pathogenic

aeromonads (*Aeromonas jandaei*) enterics (*Escherichia vulneris*; *Yersinia intermedia*) and vibrios (*V. furnissii*; *V. logei*) over recent years. With the rapid increase in the number of new vibrio taxa since 2000, it is apparent that many (e.g. *V. coralliilyticus*) have implications for the health of aquatic animals.

Fish vaccines – progress and perspectives

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Abstract not received

Infectious salmon anaemia virus – an 'influenza' virus of fish

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Infectious salmon anemia (ISA) is a disease in farmed Atlantic salmon (*Salmo salar* L.) responsible for major losses in Norwegian, Scottish, Canadian and American fish farms. ISA-virus (ISAV), the causative agent of ISA, has been suggested to represent a fifth genus within the *Orthomyxoviridae*, and genetic evidence as well as biochemical, physiochemical and morphological properties of the ISAV support this. This presentation will summarize the taxonomic and biochemical properties classifying this virus as an orthomyxovirus, and discuss the present knowledge regarding the structural and functional properties of the encoded proteins as compared to the influenza viruses.

Four major structural proteins have been recognized in purified ISAV particles, including the matrix (M1; 22–24 kDa), the nucleoprotein (NP; 66–71 kDa), and two membrane glycoproteins. The receptor-binding and receptor-destroying activities are associated with the 42 kDa glycoprotein encoded by segment 6, termed the hemagglutinin-esterase (HE). This gene contains a polymorphic region which has been suggested to be important for the determination of the pathogenicity of this virus.

Glycoproteins of infectious salmon anaemia virus

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Although the overall morphological and physicochemical properties of the ISAV are similar to those of the other orthomyxoviruses, ISAV is distinct from the influenza viruses in a number of ways. ISAV infects fish, and has an optimal replication at 15°C. It is structurally unique in having several homologous proteins that bear no significant similarity in primary sequence to those of the other orthomyxoviruses, and its genomic organization differs in several ways. Particularly, the organization of the activities associated to the ISAV surface proteins is different from that of the other orthomyxoviruses.

Influenza A and B viruses have eight gene segments and contain separate attachment and receptor-destroying envelope glycoproteins (HA and neuraminidase (NA), whereas ISAV contains one glycoprotein providing both of these functions (HE) and a separate F protein. This is the first separate orthomyxovirus fusion protein (F) described, and to our knowledge, the first pH dependent separate viral F protein described. In influenza A and B viruses the fusion activity is located to the attachment protein (HA). Influenza C viruses contain only

a single glycoprotein providing all of these functions (hemagglutinin-esterase-fusion, HEF), as reflected in the genome by the concomitant loss of one RNA segment.

This presentation will summarize the functional properties of the ISAV glycoproteins.

The development of nucleic acid vaccines against virus infections of fish

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The use of DNA vaccines offers considerable potential for immunization against virus infections of fish, DNA plasmids being easy and economical to produce and known to stimulate both antibody and cell-mediated responses in mammals. DNA immunogens expressing the G protein protect against fish rhabdovirus infections are particularly effective. Several DNA constructs containing the spring viraemia of carp virus (SVCV) glycoprotein (G) gene have been optimized for their ability to induce protection against SVCV following injection into myofibres. Antibody responses can be induced preferentially by plasmids with either full-length or truncated G constructs under the control of the cytomegalovirus (CMV) promoter; or by fusion with a second gene to maximize G protein expression. In contrast, T-cell like responses can be preferentially induced using either the simian virus 40 promoter or a fish muscle-specific promoter; or the CMV-Intron A promoter in plasmids with or without CpG motifs. The latter gives the greatest level of protection against SVCV in challenge experiments using carp. These results suggest that a Th1-like immune response is necessary for conferring protection against infection, thus confirming the potential for DNA fish vaccines against other economically important fish diseases.

The fish larval intestine – a battleground for pathogens and probionts?

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Turbot, ***Scophthalmus maximus***, Atlantic halibut, ***Hippoglossus hippoglossus***, and Atlantic cod, ***Gadus morhua***, larvae are fed rotifers, ***Brachionus plicatilis*** and/or ***Artemia*** spp. enriched on different commercial media. In addition to traditional cultivation assays, DGGE-protocols have been applied in order to circumvent cultivation of bacteria, and provide an overview of the microbiota associated with fish larvae or live feed organisms. Bacteria showing high sequence homology to the ***Listonella anguillarum*** are abundant in cod larvae. Different enrichment media have major impact on the concentration of bacteria as well as the composition of the microbial population in the enrichment cultures as well as in the larval gastrointestinal tract. In order to elucidate the ***in vivo*** effect of pathogens and tentative probionts, immunohistochemical assays have been developed, thereby labelling the different bacteria in live feed organisms, on the epithelial surfaces of larvae or in the gastrointestinal lumen of larvae. Whereas most opportunistic ***Vibrio*** spp. appear to be dependent on an oral route of administration, causing enteritis, ***Listonella anguillarum*** induces mortality following bath challenge, and adheres to the larval surfaces.

Bactericidal killing mechanisms in the lesser spotted catshark (*Scyliorhinus canicula*)

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The immune system of cartilaginous fish is poorly studied, especially in terms of leucocyte killing mechanisms for microbes. Recently we have cloned the iNOS gene (coding for the

enzyme responsible for production of nitric oxide) from the lesser spotted catshark (*Scyliorhinus canicula*), and here study its expression upon bacterial exposure. At 24 and 48 h post *in vivo* challenge with *V. anguillarum* (serogroup 01, 10^8 bacteria/fish) iNOS expression was enhanced in the spleen and was seen to increase significantly with time (P=0.039, ANOVA). However, no significant increase in iNOS expression was seen in either the gill or leydig organ. Significant expression of iNOS was also observed in isolated splenocytes stimulated with LPS (4 h incubation, P<0.005) or Poly I:C (24 h incubation, P<0.005) compared to unstimulated cells. We also demonstrate the ability of catshark blood leucocytes to undergo a respiratory burst, producing reactive oxygen species (ROS), following activation with PMA, using a ferricytochrome C colour change assay.

Session 4 Workshop – Microbiology education: dead or alive?

Provision of the education and training of the future generation of microbiologists is a subject of international concern. Is microbiology losing ground in educational and research opportunities? The meeting will offer an international exchange of views and hopefully identify some ways forward. A report of the discussion will be available after the meeting.

Posters – Abstracts

JSM 01 Genotyping of *Campylobacter* from broilers and broiler farm environment by AFLP

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Poultry meat is a risk factor for human campylobacteriosis. Minimizing the number of *Campylobacter*-positive flocks at farm level will reduce the prevalence of contaminated broiler meat and thereby improve food safety. Knowledge on the occurrence of *Campylobacter* in the environment is necessary to reveal transmittance routes to broilers. The occurrence of *Campylobacter* in broiler and outdoor environments was investigated and isolates compared in order to suggest possible routes for transmittance. Genetic fingerprinting was performed with amplified-fragment length polymorphism (AFLP).

The proportion of *Campylobacter*-positive samples varied between farms, according to implementation of bio-security measures. A high genetic diversity with some stability was found among outdoor isolates and a lower genetic diversity was found among isolates from broilers. Broilers on the farm with poorest bio-security got infected first, and had highest genetic diversity among the *Campylobacter* isolates. Broiler isolates were found in neighbouring broiler flocks and the outdoor environment, but not inside the broiler house prior to infection. Massive occurrence of *Campylobacter* with high genetic diversity suggests water, dogs and wild birds as *Campylobacter* reservoirs.

JSM 02 Genotyping of *Campylobacter* from broiler slaughterhouse by AFLP

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Campylobacteriosis is an important food-borne enteric infection. Poultry can commensally carry large amounts of *Campylobacter* that can be transmitted to the carcass during slaughter. Infected broilers entering the slaughter line cause massive contamination of the abattoir environment. In the present study, the amount and diversity of *Campylobacter* on the carcasses and in the abattoir environment during slaughter of an infected broiler flock and after washing was investigated. Genetic fingerprinting was performed using amplified-fragment length polymorphism (AFLP).

Both broiler carcasses and the abattoir environment were heavily contaminated with *Campylobacter* during processing of an infected broiler flock. *Campylobacter* remained in the environment after washing and were detected the following morning. During slaughtering of the infected broiler flock, isolates from the monitored flock dominated in the abattoir environment. The *Campylobacter* isolates detected the following morning showed a higher genetic diversity than during slaughtering of the infected flock. This may be due to a masking of the true *Campylobacter* flora by massive supply of one clone during the slaughtering of an infected flock.

JSM 03 The potential of enzybiotics against Gram-positive pathogens

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Lysostaphin is a promising 'enzybiotic' that consists of a glycyl-glycine endopeptidase and targeting domains and is actrive against MRSA. Either domain when expressed independently retains its function which offers the promise of developing novel chimeric enzybiotics. Mutational analysis of the two domains has been facilitated by a novel endopeptidase FRET assay.

We have designed a novel protein FRET substrate (MV11) that contains a pentaglycine target sequence for the endopeptidase activity of lysostaphin. Mass spectrometer analysis demonstrated that cleavage of the pentaglycine target sequence in MV11 occurred between position 2 and 3. Lysostaphin producing strains are resistant to killing by lysostaphin due to the incorporation of serine residues at position 3 and 5 of the pentaglycine linker in their cell walls. The MV11 substrate was engineered to introduce a serine residue in turn at all five positions of the pentaglycine target site to investigate the effect on cleavage and provides a model system to study resistance to lysostaphin.

JSM 04 Antimicrobial resistance of *Campylobacter* isolates from exclusively reared chickens

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Campylobacter was isolated and enumerated during the rearing cycle of free-range (56 days) and organic chickens (73 days) at 3-day intervals from hatching until slaughter. *Campylobacter coli* were the predominant *Campylobacter* species isolated from both organic and free-range chickens. Pulsed field gel electrophoresis (PFGE) of *Sma*I-digested genomic DNA from these strains revealed 16 different macro restriction patterns (MRPs) in the organic flock and 9 different PFGE MRPs in the free-range flock. *Campylobacter* isolates (n≥5). Representative of each of the MRPs were tested for their susceptibilities to 12 antibiotics prescribed in human and veterinary medicine. No variation in antibiotics sensitivity was evident between *Campylobacter* isolates of the same MRP. There was a marked difference in the frequencies of antibiotic resistance between the two flocks. Multiple antibiotic-resistant types were all but absent in organic birds but observed in the free-range flock, irrespective of the *Campylobacter* species despite the fact antimicrobials as growth promoters have been banned in the EU since 1998 and no veterinary treatment was prescribed. The range of MRPs and antibiotic resistance profiles would suggest that the free-range flock in particular had been exposed to diverse campylobacters from the environment that had either persisted within the flock from a time when antibiotic intervention was appropriate or were acquired from another source.

JSM 05 A new method for the rapid purification of FanC, the major subunit of K99

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Several strategies and methods have been attempted to purify K99 fimbriae from enterotoxigenic *Escherichia coli* strains. Most of these methods are technically complex and time consuming. We have developed a single step ion-exchange chromatography method that takes just a few hours for purification of this important virulence factor.

K99 fimbriae were stripped from *E. coli* B41 by heat treatment and phosphate urea buffer. The crude extracts were then equilibrated with tris buffer and were loaded to a HiTrap SP-XL column. By step-wise elevation of sodium chloride concentration, the FanC was eluted as a

single species. The purified protein agglutinated sheep and horse red blood cells and had high activity with anti-K99 monoclonal antibody in ELISA. Also, pre-incubation of red blood cells with the purified protein blocked adhesion of B41 bacteria. LPS contamination was undetectable using a silver staining method.

In summary, this simple, inexpensive method yields purified FanC that has good purity, stability and biological activity, making it suitable for many purposes including vaccination.

JSM 06 Immune responses of calves after vaccination with live attenuated *aroA* mutant of *Pasteurella multocida* B:2

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Haemorrhagic Septicaemia (HS) is an acute disease of cattle and buffaloes in tropical countries characterized by terminal septicaemia and high mortality. The causative agent, *Pasteurella multocida*, is a Gram-negative coccobacillus and only serotype B:2 is associated with the disease in Asia. HS is controlled by vaccination but existing vaccines are killed organisms adjuvanted with oil or aluminium and also have problems such as short duration of protection and difficult administration. JRMT12, an *aroA* mutant of *Pasteurella multocida*, constructed previously in our laboratory, attenuated for virulence in the mouse and protects mice from challenge with the virulent strain. Another experiment showed that JRMT12, given intramuscularly as two doses, causes strong protective (100%) immunity against challenge. In this work, the safety and potency of intramuscular vaccination of calves with different doses (10^7, 10^8 and 10^9 c.f.u.) of JRMT12 was tested. Results showed, all of the vaccinated animals survived challenge and all the control animals were killed due to challenge. IgG titres, showed a marked increase only after second vaccination in all groups with a good relationship between ELISA titres and doses of vaccine. Immunoblot revealed four major immunogenic bands in immune calves sera. Peripheral blood mononuclear cells (PBMCs) of challenged calves showed suppression in the potential respond to mitogenic effects of concanavalin-A (con-A). *In vitro* experiments using envelope extract of *Pasteurella multocida* showed suppression of the PBMCs to respond to con-A.

JSM 07 Genital ulcer disease and herpes simplex virus infection in Tanzanian STD patients

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Genital infection with herpes simplex virus type 2 (HSV-2) is one of the major sexually transmitted diseases (STDs) both in developing and in developed countries, and it is becoming increasingly important. Several reports indicate that that there is an association between HSV-2 and HIV infections, and one of several possible explanations is an increased access of HIV through damages in the mucosa caused by genital ulcers.

We have studied the prevalence of HSV-2 antibodies among 1,095 STD patients and 481 non-STD participants (pregnant women, blood donors and medical students) in Tanzania and in Norway. HSV-2-specific antibodies were detected using a non-commercial ELISA method

based on a branched oligopeptide (peptide 55) (1, 2). Furthermore, specimens from genital ulcers in 321 consecutive Tanzanian STD patients were examined by PCR to detect DNA from *Treponema pallidum*, *Haemophilus ducreyi*, HSV-1 or HSV-2.

A seropositivity of 70% (3) among Tanzanian STD patients represents a dramatic increase from 43% observed 5–10 years earlier (4). Higher seroprevalence was associated with female gender, increasing age, previous STDs and HIV positivity.

In swabs from genital ulcers no pathogen was detected in 29% of the cases. HSV-2 was identified in 65% of the swabs, HSV-1 in 7%, *Treponema pallidum* in 3% and *Haemophilus ducreyi* in 4%. A single pathogen was most common, as it was present in 64% of the samples. Dual infection was diagnosed in 22 samples (7%), and three pathogens detected in one single ulcer. In 7 of 22 HSV-1-positive swabs, HSV-1 was the only pathogen. The results show that HSV-2 is the predominant cause of genital ulcers among Tanzanian STD patients, and HSV-1 is as least as common as the agents causing syphilis and chancroid.

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JSM 08 Antibodies against HSV-1 and HSV-2 in children and young persons attending an outpatient clinic in Tanzania

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Primary infections with herpes simplex virus type 1 (HSV-1) usually occur in the young child and are often asymptomatic. In contrast, herpes simplex virus type 2 (HSV-2) is mainly sexually transmitted, and most primary HSV-2 infections do consequently occur later in life. Antibodies against HSV-1 are more prevalent than against HSV-2.

Reports from the African continent indicate that the percentage of HSV-2 infected adults has increased in recent years. This is alarming, not least becasuse of the association between infections with HSV-2 and HIV. However, little is known about HSV infections among African children and young persons. We have analysed more than 500 sera from children and teenagers attending an outpatient clinic for acute diseases in Dar es Salaam, Tanzania, using methods that discriminate between HSV-1 and HSV-2 antibodies. The methods were an in-house Western blot which was evaluated against a commercial Western blot, and two ELISA methods in which the antigenic oligopeptide was specific for HSV-1 or HSV-2 (1,2,3). The prevalence of HSV-1 antibodies was nearly 70% in the age group 1–4 years, increasing to about 90% in the group 17–20 years. Depending upon the method, between 14 and 20% in age group 1–4 years had HSV-2 antibodies. The detected antibodies in this age group could include maternal antibodies, since the figures decreased to 10–14% in age group 5–8 years. Thereafter the HSV-2 prevalence increased continuously to reach approximately 40% in the age group 17–20 years. The high percentage of HSV-2 infection in children and young people was unexpected. Possible reasons will be discussed.

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JSM 09 Identification of N-terminal peptides by MALDI-TOF of processed proteins secreted through the general secretory pathway

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Mycobacterium tuberculosis is the causative agent for tuberculosis which is a leading cause of death of infectious diseases in the world with almost 3 million casualties each year. We are investigating the proteome of *M. tuberculosis* with the specific aim to identify exported proteins using culture filtrates of 3 week old cultures of *M. tuberculosis* on the wholly synthetic Sauton medium. Culture filtrate proteins are separated by 2-D PAGE and trypsinized spots are subjected to MALDI-TOF analysis. When searching the databases using the mass-spectra from MALDI-TOF, the N-terminal peptide will generally not be identified because the mature protein is usually not represented in the public databases. For optimization of the identification of exported proteins we have made a database of truncated proteins removing all predicted signal peptides. We will show that this approach enhances the possibility of obtaining significant hits when using the Mascot program. In this way we also obtain experimental evidence for predicted signal peptidase cleavage sites.

JSM 10 Predicting proteins exported through the general secretory pathway in *Mycobacterium tuberculosis* H37Rv

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The Neural Network method (SignalPNN) and the Hidden Markov Model (SignalPHMM) for predicting signal peptides are publicly available at the SignalP server, and are primarily designed for predicting signal peptides in Eukaryotic organisms, Gram-positive or Gram-negative organisms. Mycobacteria are not classified as Gram-positive or -negative, but it has been shown that signal peptides of mycobacteria are most closely related to Gram-positive signal peptides. An important question is whether the SignalPNN and SignalPHMM methods can be used for prediction of mycobacterial signal peptides. To investigate this we used two sets of characterized *M. tuberculosis* proteins with and without signal peptides, mainly as determined by N-terminal sequence determination of native proteins. A limited set of mycobacterial secreted proteins with signal peptides have been identified by N-terminal sequence analysis to confirm the cleavage site. In addition, there is now information available on a fairly large number of native proteins with N-terminal sequences that start at, or very close to the predicted translational start site. These two sets of *M. tuberculosis* proteins were used to validate the SignalPNN and SignalPHMM methods for use with *M. tuberculosis*. The results showed that the SignalPHMM method is superior ot the SignalPNN method for predicting proteins secreted via the general secretory pathway in *M. tuberculosis*.

JSM 11 Expression of *Mycobacterium bovis* antigenic proteins MPB70 and MPB83 is controlled by the alternate sigma factor σ^K

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Mycobacterium bovis Bacille Calmette-Guérin (BCG) strains are genetically and phenotypically heterogeneous. Expression of the antigenic proteins MPB70 and MPB83 is known to vary considerably across BCG strains, however, the reason for this phenotypic difference has remained unknown. By immunoblot, we separated BCG into high- and low-producing strains. By quantitative RT-PCR, we determined that transcription of the antigen encoding genes, *mpb70* and *mpb83*, follows the same strain pattern with mRNA levels reduced over 50-fold in low-producing strains. Transcriptome comparison of the same BCG strains by DNA microarray revealed two gene regions consistently down-regulated in low-producing strains compared to high-producing strains, one including *mpb70* (Rv2875) and *mpb83* (Rv2873) and a second that includes the predicted sigma factor, σ^K. DNA sequence analysis revealed a point mutation in the start codon of *sigK* in all low-producing BCG strains. Complementation of a low-producing strain, BCG Pasteur, with wild-type *sigK* fully restored MPB70 and MPB83 production. Microarray-based analysis and confirmatory RT-PCR of the complemented strains revealed an up-regulation in gene transcription limited to the *sigK* and the *mpb83*/*mpb70* gene regions. These data demonstrate that a mutation of *sigK* is responsible for decreased expression of MPB70 and MPB83 in low-producing BCG strains and provide clues into the role of *Mycobacterium tuberculosis* σ^K.

JSM 12 Crystal structure of low-molecular-weight protein tyrosine phosphatase from *Mycobacterium tuberculosis* at 1.9 Å resolution

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The low-molecular-weight protein tyrosine phosphatase (LMWPTPase) belongs to a distinctive class of phosphotyrosine phosphatases widely distributed among prokaryotes and eukaryotes. We report here the crystal structure of LMWPTPase of microbial origin, the first of its kind from *Mycobacterium tuberculosis*. The structure was determined to be two crystal forms at 1.9- and 2.5-Å resolutions. These structural forms are compared with those of the LMWPTPases of eukaryotes. Though the overall structure resembles that of the eukaryotic LMWPTPases, there are significant changes around the active site and the protein tyrosine phosphatase (PTP) loop. The variable loop forming the wall of the crevice leading to the active site is conformationally unchanged from that of mammalian LMWPTPase; however, differences are observed in the residues involved, suggesting that they have a role in influencing different substrate specificities. The single amino acid substitution (Leu12Thr) in the consensus sequence of the PTP loop, CTGNI CRS, has a major role in the stabilization of the PTP loop, unlike what occurs in mammalian LMWPTPases. A chloride ion and a glycerol molecule were modeled in the active site where the chloride ion interacts in a manner similar to that of phosphate with the main chain nitrogens of the PTP loop. This structural study, in addition to identifying specific mycobacterial features, may also form the basis for exploring the mechanism of the substrate specificities of bacterial LMWPTPases.

JSM 13

ETS transcription factors in prostate cancer

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ERG (*ETS* related gene) is one of more than 30 transcription factors of the ETS family. The founding member ETS-1 was discovered in a transforming retrovirus designated E26 after its isolation from chicken with erythroblastosis in 1962. E26 contained parts of the oncogene *myb* fused to a novel oncogene whose cellular counterpart was named c-*ETS-1* (E26 transformation-specific sequence) The defining feature of ETS transcription factors is a conserved DNA binding domain that recognizes the promoter sequence GGA$^{A}/_{T}$. A subset of ETS family proteins, including ERG, contains the conserved PNT aminoterminal domain that forms a helix-loop-helix structure for protein interaction. The ERG/FLI-1 subfamily has a putative COOH-terminal activation domain in addition to the aminoterminal one. In Ewing's sarcoma *ERG* and *FLI-1* DNA-binding domains are involved in defining translocations with the activation domains of RNA-binding proteins like EWS and FUS. The ERG-FUS is also found in t(16;21)(p11,q22)-positive acute myelogenous leukemia (AML). In AML other *ETS* transcription factors are involved in different translocations. Overexpression of *ERG* due to gene amplification has been reported in AML. Since *ERG* is expressed during embryonal differentiation of the urogenital tract followed by downregulation, the abundant overexpression presently reported in prostate cancer may be highly relevant for the further understanding of prostate carcinogenesis.

Prostate carcinoma is the most common cancer of western men and is a markedly heterogeneous disease. The aim of this study was to identify signatures of differentially expressed genes in prostate cancer using DNA microarray technology, evaluating expression profiles in matched pairs of benign and malignant tissue. Samples were initially collected from 33 radical prostatectomies, and 52 specimens were included, representing 29 histologically verified primary tumours, 19 paired samples of malignant (T) and benign (B) tissue, and 4 non-paired benign tissue samples. Microarray analysis was performed using a sequence verified set of 40,000 human cDNA clones. Several transcription factors were found among the genes most consistently and highly upregulated in prostate cancer tissue compared to paired benign tissue. Among these transcription factors *ERG* was selected for further validation. Tumour/benign (T/B) expression ratios exceeding 100 fold were found in several cases according to real time quantitative PCR. In an expanded patient series altogether 13 of 31 matched pairs of T and B exhibited more than 20 fold T/B ratios. The *ERG1* isoform was most abundantly overexpressed and contributed most to the very high *ERG* expression in cancer. Real time quantitative PCR was also used to examine the expression of other transcription factors, including other ETS family members, SIM2 and HOX transcription factors.

Our hypothesis is that ERG overexpression may change the regulation of an entire gene expression module – possibly with similar promoter motifs – for the benefit of increased cellular proliferation.

Analysis of gene expression using DNA microarrays is valuable for the hypothesis-independent discovery of gene expression and next leads to specific hypothesis regarding molecular carcinogenesis. It also leads to selection of prognostic markers of potential clinical use.

JSM 14 The humoral immune response and protective efficacy of vaccination with inactivated split and whole influenza virus vaccines in BALB/c mice

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Recently the urgency of developing a pandemic influenza vaccine has lead to the re-evaluation of the use of whole virus vaccine. In this study we have compared the kinetics and the magnitude of the humoral immune response to two different influenza vaccine formulations, whole and split virus vaccines and examined their protective efficacy upon homologous viral challenge.

BALB/c mice were immunized intramuscularly with 1 or 2 doses (3 weeks apart) of three different strengths (7.5, 15 or 30µg) HA of monovalent A/Panama/2007/99 (H3N2) split or whole virus vaccine and subsequently challenged intranasally with A/Moscow/10/99 (H3N2) influenza virus (200 MID_{50}) 9 weeks after the last dose of vaccine. Mice were sacrificed at various time intervals after vaccination and viral challenge. The antibody secreting cell (ASC) response was examined in the spleen, bone marrow and lungs (challenged mice only) using the Enzyme-Linked ImmunoSpot Assay (ELISPOT). Sera were collected at the time of sacrifice and analysed by ELISA, haemagglutination inhibition and virus neutralization assays. Nasal wash samples were collected for the initial four days after challenge and the level of replicative virus quantified.

After vaccination, the two vaccine formulations induced similar kinetics of the ASC response. Whole virus vaccine was more immunogenic generally eliciting higher numbers of systemic ASC and a quicker and higher neutralizing antibody response, particularly after the first dose of vaccine. Interestingly, the two vaccines induced different IgG subclass profiles. Split virus vaccine stimulated both IgG1 and IgG2a antibodies suggestive of mixed Th1 and Th2 response, whereas whole virus vaccine induced mainly an IgG2a antibody response, which is indicative of a dominant Th1 response. This was also confirmed with the cytokine profile from stimulated spleen cells, whole virus vaccine induce Th1 type cytokines, whereas split virus vaccine induce more Th2 type cytokines.

Upon viral challenge, both vaccine formulations reduced the level and duration of viral shedding (by at least two days) compared to unvaccinated controls. Mice immunized with one dose of whole virus vaccine more effectively reduced viral shedding than those vaccinated with one dose of split virus vaccine. However, after the second dose of vaccine, viral replication was most effectively limited in mice vaccinated with split virus vaccine, particularly 15 and 30µg strengths and this was correlated with high influenza specific serum IgG concentrations. When mice were grouped according to the peak nasal wash titre, mice with no or low viral shedding had significantly higher pre-challenge serum IgG concentrations than mice with higher viral shedding in the nasal cavity.

Thus, in a non-lethal upper respiratory tract challenge model serum IgG was important in reducing viral replication.

JSM 15 The unusual epidemiology of *Mycobacterium bovis* in buffalo in Iran

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Mycobacterium bovis is the cause of bovine tuberculosis (bovine TB) in animals and humans although cattle are the main host. Buffalo can also be infected and develop bovine TB. In

Iran, almost half a million buffalo are farmed, mainly in three provinces. In West Azerbaijan, which has the largest numbers of buffalo, cattle and buffalo are often farmed together. However, according to veterinary records over the last 25 years, there have been no reports of bovine TB in buffalo although the disease is often reported in cattle in this province.

Eighteen and 140 pathology specimens were cultured from cattle and buffalo respectively from West Azerbaijani abattoirs. No *M. bovis* was recovered from the buffalo specimens but the pathogen was isolated from 13 cattle. Spoligotyping showed a relatively higher polymorphism within these isolate compared to the other Iranian provinces. We believe more studies are required to understand the epidemiology of *M. bovis* in the Iranian buffalos.

JSM 16 Diversity of microbial communities in sponges from Scottish coastal waters

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Marine sponges have become important organisms of study in the search for novel microbial products. However, some novel bioactive compounds have recently been shown to be released by sponge associated bacteria rather than by the sponge cells. The aim of this study was therefore to investigate the diversity of the bacterial community within sponges from Scottish Coastal waters, since most studies have so far focused on tropical sponges. Sponge species studied were *Suberites domuncula*, *S. carnosus*, *Halichondria panicea*, *Clione celata* and *Pachyimatisma johnstonii*. We have obtained 1,014 isolates from these sponges which were then classified on the basis of partial 16S rDNA sequences and 107 of these were identified as members of the α- and γ-*Proteobacteria*. An air membrane surface (AMS) bioreactor (1) and a Reacyn, Bioreactor were used to study the production of antimicrobial compounds by these bacteria. Supernatants from sponge isolates of *Bacillus cereus*, *B. subtilis* and *B. licheniformis* contained antimicrobial compounds active against other marine bacterial isolates. These studies demonstrate the biotechnological potential of Gram-positive bacteria from temperate sponges.

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JSM 17 Microbial genotyping by combinatorial probing; a bioinformatics and reverse line blot approach

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Blood Stream Infections (BSIs) are a major health problem across the world with over 96% of cases caused by 30 bacteria and fungi. Presently, lengthy culture methods are used for microbe identification. Alternatively, rapid diagnosis using sequence identification methods can be used to enable timely and effective antibiotic prescription. Multiple probes can be patterned on a solid surface for the identification of any of the target microbes in a single test.

The ribosomal RNA small subunit sequence (16S for bacteria or 18S for fungi) is present in high copy numbers per cell, presenting the possibility for identification without fragment amplification. The variation within the molecule is limited due to size and functional constraints and a simple one probe one target strategy is insufficient for accurate species identification. To address this problem, bioinformatic analysis has been used for multiple sequence alignments to identify conserved and variable regions. This sequence information has been used for the development of an accurate combinatorial probing strategy.

The molecular specificity of the probe panel has been validated by reverse line blotting, a high throughput 2-D hybridization technique akin to the Southern blot method. Here, multiple samples can be simultaneously interrogated with all probes. Further work entails optimization of the RLB assay for the identification of clinical isolates and refinement of the probe panel by fully comprehensive database analysis.

JSM 18 Bacteraemia caused by *Actinobaculum schalii*

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Background *Actinobaculum* spp. are curved Gram-positive, not acid-fast, non-sporing rods, which may exhibit branching, and are non-haemolytic on blood agar. *Actinobaculum* is related to *Actinomyces*. In MEDLINE (as of July 1, 2005) there were 11 hits for '*Actinobaculum*' alone and 4 for '*Actinobaculum schalii*'. A case of pyleonephritis caused by *A. schalii* has been described – among other conditions. Strains of this bacterium have been found in blood and urine previously. The NIPH has from 2001 to 2005 sequenced five samples containing samples containing *A. schalii*. No cases have – to our knowledge – as yet been published.

Materials/methods The blood cultures which proved to be culture-positive for *Actinobaculum schalii* were subjected primary tests at our laboratory. The NIPH performed the sequencing and reference testing at a central level.

Results A blood culture from a 91 year old man with septicaemia was cultivation-positive at laboratory showing growth of a Gram-positive bacterium later identified to *Actinobaculum* to the genus level by 16S rRNA sequencing. The traditional tests included:

Growth: blood agar, aerobic conditions: none	Same agar, anaerobic conditions: +
Growth: chocolate agar, aerobic conditions: none	Same agar, anaerobic conditions: +
Growth: lactose agar, aerobic conditions: none	Anaerobic agar, anaerobic conditions: +
Metronidazole 10 µg: Resistant	Corrosion: –
Vancomycin: Sensitive	Motility at 30°C: –
Catalase: –	OF Glucose: No growth
Oxydase: –	

Discussion In Norway bacteraemia caused by *A. schalii* has not – to our knowledge – been published before. This is one of the few case reports in MEDLINE's history demonstrating *A. schalii* as the cause of a systemic infection such as bacteraemia/septicaemia.

Conclusion A medium-sized microbiological department, such as ours – may encounter rather unusual findings. Standard diagnostic tests may be rather efficient. Strengthening of the diagnostic capability on a county/regional level may be indicated.

JSM 19 Identification of bacteria in blood samples from newborns with signs of sepsis using broad range 16S rDNA PCR without prior cultivation

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Approximately 10% of all newborns at Akershus University Hospital are transferred to the neonatal intensive care unit (NICU) for further observation and treatment. Of 3,627 children born during the year 2004, 367 were transferred to the NICU, and 133 were treated for suspected sepsis. The average length of treatment was 6.9 days.

The clinical signs of serious infection in newborns are unspecific, and the microbiological identification of the causative agent is difficult. In less than 20% of the patients with clinical signs of sepsis an infectious agent is identified by conventional blood culture methods. A negative blood culture does not necessarily imply that there are no bacterial causes to the clinical manifestation. The sepsis may be caused by bacteria that are difficult to cultivate, or there are to few viable bacterial cells in the samples to be identified by conventional methods. In addition, the signs of sepsis could be related to non-infectious causes.

We have investigated the use of broad-range 16S rDNA PCR as a cultivation-independent approach for identifying bacteria in blood samples taken from newborns with clinical signs of sepsis. To establish the sensitivity of the method, EDTA blood samples from a healthy donor were spiked with live *Staphylococcus aureus* and *E. coli* at known concentrations (cell forming units, c.f.u.). The spiked blood samples were divided in plasma and cell fraction and stored at –70°C. Nucleic acid was extracted from 200µl of both fractions using the QIAamp DNA Blood kit (Qiagen). 10% of the extracted DNA was analysed for the presence of bacterial 16S rDNA using 35 to 40 cycles of PCR with reagents tested to reveal no signs of contaminating DNA at the given conditions. The identity of the PCR products was analysed by DNA sequencing. A total of 48 patient samples were collected, treated and analysed in the same manner. Analyses of the patient samples were performed in a blinded fashion with no knowledge to patient identity or the result of the blood culture.

Detection limit for *S. aureus* and *E. coli* was 10^3–10^4 c.f.u./ml blood. *S. aureus* was detected at highest sensitivity in the plasma fraction and *E. coli* in the cellular fraction. As bacterial DNA rather than living pathogens are detected by PCR, amplification of small amounts of bacterial DNA contaminants present in reagents and laboratory equipment was not unexpectedly found to be a major complicating factor. In addition, batch variation of reagents was found to be rather large. The results of this work will be presented.

JSM 20 Validation of a restriction dependent PCR method for virus detection in human cerebrospinal fluid (CSF)

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Exogenous infectious agents or activation of endogenous retroviral sequences may be involved in the etiology of central nervous system diseases. We have compared the PCR results from routine diagnostic procedures and results from scanning electron microscopy

with a new model system using restriction-dependent PCR. DNA and RNA are isolated from DNase treated CSF. DNA and cDNA are digested with *Csp*6I and *Eco*RI. A subsequent ligation of sequence-specific restriction-site adaptors followed by PCR amplification with adaptor specific primers is then carried out. Remaining nucleic acids are amplified and sequenced. The method is developed in a model system using known DNA and RNA viruses [Herpes simplex virus (ds DNA), Rotavirus (dsRNA)] and the plasmid vector pBR322. A report of this application for virus detection in human samples will be presented.

JSM 21 The role of defensins in controlling *Salmonella* infection in chickens

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Poultry are the major source of *Salmonella enterica* infections for humans in Europe. Salmonellosis in chickens can be induced either by invasive *Salmonella* such as *S.* Typhimurium or restricted host range serotypes such as *S.* Gallinarum. In this study, the expression of Gallinacins 1α, 2, 3 and 9 in line N and line 6_1 chickens, both resistant to salmonellosis but responding differently to *Salmonella* colonization, have been analysed using real-time RT-PCR with Taqman analysis. Gallinacins are the chicken's β-defensins, which are expressed by heterophils (the avian neutrophil equivalent) and some epithelial cells.

S. Typhimurium persists in the caeca of line N chickens only. Gallinacin 3 was constitutively expressed in the caeca and caecal tonsils and Gallinacin 9 in caecal tonsils of line 6_1 chickens. However, both Gallinacins were inhibited after infection of line N chickens. These results suggest that these Gallinacins might play a role in the differential responses to *S.* Typhimurium colonization observed in the two lines.

S. Gallinarum persists in the caeca of both lines. However, *S.* Gallinarum suppresses Gallinacin 3 expression in the caeca and caecal tonsils of line N chickens, as after *S.* Typhimurium infection, while it inhibits the expression of this defensin only in the caeca of line 6_1 chickens. Therefore, *S.* Gallinarum may suppress Gallinacin 3 expression allowing it to persist in the chicken's gut.

JSM 22 Matrix assisted laser desorption/ionization time-of-flight mass spectrometry provides fast and reliable identification of *Staphylococcus haemolyticus*

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Introduction Staphylococcus haemolyticus is an emerging pathogen more resistant to antibiotics than most MRSA. It causes symptoms indistinguishable from *Staphylococcus aureus* infections and is responsible for some outbreaks in orthopaedic and neonatal wards mimicking MRSA infection. Analysis of these isolates can mistakenly be perceived and identified as low-grade pathogenic coagulase-negative staphylococci. However, a fast and reliable identification is needed in order to institute appropriate antibiotic therapy. To achieve this we have successfully used the 'Peterborough Collection' of clinical isolates to establish a MALDI-TOF MS database for *S. haemolyticus*.

Methods Intact *S. haemolyticus* cells are transferred from a culture plate to a MALDI target plate and overlaid with the MALDI matrix 5-chloro-2-mercaptobenzothiazole. The co-crystallized sample is then irradiated with a N_2 laser and the resulting plume of positive

ions separated using time-of-flight mass spectrometry. This produces a characteristic mass spectral fingerprint pattern, which forms the basis of identification against a database containing representative spectra of the species. The MALDI-TOF MS technique also allows for high sample throughput and rapid identification of isolates, since the collection and analysis of spectral data against a substantial database requires ~1.5 hours for a 96-well MALDI target plate.

Conclusion In this study 71 (93.4%) of the 76 clinical strains of *S. haemolyticus* were identified correctly against a database of more than 3,600 spectral entries representing over 500 different bacterial species. Furthermore, conclusive identification was confirmed for the majority of these strains since all 8 top matches were correct to species. Comparison of three misidentified test spectra with their corresponding spectral matches demonstrated unique high mass ions exclusive to the matching of *S. haemolyticus* spectra. Therefore filtering the matches for these ions it is possible to correctly identify 74 (97.4%) of the 76 clinical strains to species level. MALDI-TOF MS has successfully identified 97.4% clinical isolates of *S. haemolyticus*, demonstrating the technique provides fast reliable identification of this emerging pathogen.

JSM 23 Microarray based detection of bacterial pathogens in COPD

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COPD is a slowly progressive and irreversible disorder characterized by the presence of airflow obstruction. Exacerbations are primarily triggered by respiratory tract infections. The gold standard for bacterial detection is culture, which lacks speed and sensitivity. Some atypical organisms are too difficult to culture. Microarrays are powerful tools that allow the simultaneous detection of thousands of bacterial genes or DNA sequences on one glass slide. Its use has been mostly in gene expression, but it can also be used for bacterial detection. The purpose of this study was to design and evaluate a DNA microarray for the detection of bacterial species in COPD. Combined PCR product and 70mer oligonucleotide arrays of respiratory pathogens were probed with flourescently labelled DNA derived from COPD sputum samples. Real-time PCR was used to confirm and quantitate the microarray results. Key pathogens such as *S. pneumoniae*, *M. catarrhalis* and *Haemophilus* species as well as atypical pathogens were identified. DNA microarrays can be applied to the identification of pathogens in respiratory syndromes.

JSM 24 Impact of a polyomavirus (BK) on gene expression in human cells analysed by microarrays

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Microarray technology allows for a global approach to examining mRNA expression. We are particularly interested in the impact of different viruses, assuming that the method may help us understand the complex interaction between virus and host cell, and possibly point to novel genes involved in antiviral defence. In the present paper we report studies on the effect of BK virus on human umbilical endothelial cells.

Virus was seeded out in sufficient number to obtain synchronous infection of a majority of the cells, as examined by antibodies detecting viral proteins. Cells were harvested 24 and 40 h after infection along with mock infected controls. The cells were immediately lysed and frozen. The viral life cycle is approximately 48 h. RNA extracts were labelled with fluorescent

dyes Cy3 (control) and Cy5 (infected cells), and hybridized on 35 k human oligo microarrays (Operon based arrays from the Norwegian Microarray Consortium). Four parallel samples were analysed for each time point.

The virus had surprisingly moderate effect on cellular transcription. Some of the genes with appreciably changed transcription levels will be discussed.

JSM 25 Biofilm production in coagulase-negative staphylococci (CoNS)

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Background CoNS are important human pathogens. Biofilm production is an important pathogenic factor in medical device associated infections. The *ica* operon and *atlE* are central genes encoding biofilm production.

Methods We investigated the occurrence of *ica* and *atlE* by PCR, Dot blot and Southern blot hybridization in 203 blood culture CoNS isolates from neonates. In addition we did semiquantitative determination of biofilm production.

We designed a flow cell model, attempting to mimic the *in vivo* environment.

Results 105 of the 203 strains produced biofilm. The combination of *ica* and *atlE* occurred in 66% of the biofilm producing strains. Conversely, 11% of the non-biofilm producers had both *ica* and *atlE*. In the flow cell model both growth and biofilm formation was continuously monitored through a microscope. However, quantification in the flow cell model requires advanced software and preferentially CLSM to optimize the method.

Conclusion ica and *atlE* are prevalent in the biofilm-forming phenotype, but not essential. The expression of the biofilm associated genes is regulated by a variety of factors which may explain discrepancies between genotype and phenotype in our study.

JSM 26 Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from blood

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Staphylococcus aureus has long been recognized as an important human pathogen, capable of causing a wide variety of infections. In the last decade proportion of infections due to methicillin-resistant *S. aureus* has increased in nosocomial and community settings. Methicillin resistance in staphylococci is caused by the expression of PBP2a encoded by the *mecA* gene, that is located on a genetic element called the staphylococcal cassette chromosome (*SCCmec*). Five types of *SCCmec* have been identified until now. Types I, II and III have been associated with nosocomial infections and types IV and V with community-acquired infections. The aims of this study were to evaluate the susceptibility profile of MRSA strains isolated from blood of patients seeking medical assistance at Hospital das Clinicas da

Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP), identify the *SCCmec* types and analyse the DNA profile in order to determine if there were predominant lineages. During one year, all MRSA blood isolates from the Microbiology Laboratory of Central Laboratory Division of HC-FMUSP were submitted to oxacillin agar screening test, antimicrobial susceptibility test by broth microdilution method, multiplex polymerase chain reaction (PCR) to detect *mecA* and *coa* genes, *SCCmec* typing and molecular typing by pulsed-field gel electrophoresis (PFGE). Eighty-nine percent of the isolates were nosocomial-acquired; 10% were associated with healthcare and only 1% were ascertained as community-acquired infection. Amid 223 isolates, 69% of strains were observed to be multiresistant; 83% had *SCCmec* type IIIA and had a predominant pattern by PFGE. Some strains nosocomial-acquired presented *SCCmec* type IV, resistance only to beta-lactams and demonstrated PFGE pattern with one predominant lineage. In conclusion, our results confirm that the predominant local MRSA strain is related to the Brazilian endemic clone and had the *SCCmec* type IIIA. Also there is an emerging lineage that carries *SCCmec* type IV. Although SCCmec type IV *S. aureus* typically is described as a predominantly community-acquired pathogen, in our setting it is clearly nosocomial.

JSM 27 Inadequate knowledge about Sexually Transmitted Diseases [STDs] and risky sexual behaviour: the risk factors widespread about STDs among youth in developing countries

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Issues This abstract shows that the low level of knowledge and risky sexual behaviour of youth are the risk factors for the high rate of spread of STDs among Nigerian youth.

Description A self developed validated and reliable questionnaire [r=0.77] was used to collect the data needed for the study and percentage was used to analyse the data. The population of the study was made up of the resident undergraduate/graduate students in male hostels in the Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. The sample size is 636 selected through simple random sampling technique. The demographic data is as followings: Out of 636 respondents, 11 were below 16 years old, 95 were between 16 and 20, 309 were between 21 and 25, and 223, between 26 years and above. The two well developed tables were constructed. Relative Risk [RR] calculated is 1.7, i.e. RR>1, indicating that the factors are risk factors, and the Confidential Interval [CI] for RR at 95% Significant level is 1.61<1.7<1.79 from the formula, CI Lower limit<RR<CI Upper limit.

Lessons learned In Table 1 which shows the knowledge of the respondents about STDs, revealed that 36.65% responses had knowledge about the diseases. Table 2 which shows the risky sexual behaviour of the respondents, revealed that the higher education students in Nigeria do engage in one risky sexual behaviour or the other, with majority of them having multiple sexual partners without using condom during the sexual intercourse, and this makes them highly prone to STDs including HIV/AIDS.

Conclusion It is clearly seen that low level of knowledge and engagement in risky sexual behaviour are the obvious risk factors for the high rate of STDs in Nigeria and most developing countries.

JSM 28 Sero-prevalence of hepatitis C virus among blood donors in Lagos, Nigeria

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Hepatitis infection is on the increase in our society with most people becoming infected at birth or in early childhood despite the routine screening for Hepatitis B virus (HBV) in blood for transfusion. Perhaps, this problem may be due to HCV because bloods were usually not screened for it. Hence, this work is designed at screening blood donors for Hepatitis C virus (HCV), one of the causative agents of viral hepatitis. One hundred and sixty-seven (167) blood samples from donors which were sero-negative to HBV markers were screened for presence of HCV IgM antibodies using a third generation ELISA kit (Diagnostic Bioprobes Srl Kit). Out of the 167 samples tested, 14 (8.4%) were positive for anti-HCV. The highest prevalence rate was recorded in the age group 30–39 years. There is a statistical significant association between the gender and the rate of infection. Prevalence rate is high (8.4%) hence, the need to improve on the screening quality of blood meant for transfusion. There is also the need to make the HCV kit available and affordable as well as develop effective vaccines against the virus.

JSM 29 Diagnosis of foot-and-mouth disease (FMD) in U.P. (North India)

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The outbreaks of FMD disrupt the animal industry, including the export of animals and animals' products. However, disease has been observed in more than 30 countries during the last one and a half years. Many workers reported outbreaks of FMD in different countries. In India (1990), the outbreak of FMD was due to serotype O and topotype Pan-Asia which spreads to Asia and than to Turkish Thrace, Greece and Bulgaria. This strain was accidentally entered even into South Africa. Then this virus was further discovered in UK. (2001).

Now there is urgent need for a test which can differentiate vaccinated from FMD affected animals. Presently ELISA, EITB, PCR etc are routinely used in FMD diagnosis from different disease situations. Different ELISA based technologies are quite useful in present scenario. The molecular epidemiology of FMD now uses nucleotide sequencing to individually identify strains of FMDV. Dendrograms can be used to establish genetic relationship between different FMD isolates. These dendrograms are routinely used to get an indication of the likely origin of new outbreak strains of FMDV. Dendrograms for all FMD serotypes are routinely updated.

A systematic epidemiological study on foot-and-mouth disease was conducted to assess geographical distribution and seasonal occurrence of different types and subtypes of the virus associated with this disease and the factors associated with the maintenance and spread of infection in selected areas of North India. For FMD virus isolation and typing work, a total of 21 specimens of vesicular epithelium were collected during this year from different outbreaks of FMD. All of these were collected from buffaloes, the main species involved during the various outbreaks while 11 of the strains were typed 'O' FMD virus.

JSM 30 Gene cloning of a P35 antigen of *Toxoplasma gondii* Rh strain existed in Iran

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Background The use of serologic tests to show specific antibodies to the protozoan parasite *Toxoplasma gondii* is the primary method of diagnosis. The currently available tests for the detection of IgG and IgM antibodies in infected individuals vary in their ability to detect serum antibodies. The differences observed between these serological tests are probably due in part to the various preparations of antigen used to detect serum antibody. Due to the inherent limitations of the whole tachyzoite antigen in serologic tests the advent of purified and tachyzoite specific recombinant antigens obtained via molecular biology is an attractive alternative for the detection of serum antibodies.

Methods A fragment of 746 bp from P35 gene of *Toxoplasma gondii* encoding P35 tachyzoite specific antigen was amplified by polymerase chain reaction (PCR). The amplified fragment was sequenced to verifying and then cloned into pBR322-T vector and subcloned into pGEMEX-1 expression vector.

Results A 746 bp band of PCR product was seen on agarose gel and the DNA fragment sequence was compared to the GenBank database using BLAST program and high scores alignment (1338) was seen between our sequence and P35antigen sequence. Recombinant plasmid was checked for the presence of the insert and for the correct orientation using restriction enzyme digestions.

Conclusion Based on sequence result there is no difference in P35 gene between RH strain existant in Iran with GenBank data.

JSM 31 Determination of plasmid profiles, antibiotic resistance and phenotypic virulent strains of *Shigella flexneri* in Tehran

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Background Shigellosis in an acute gastroenteritis caused by *Shigella* species, including *S. dysenteriae*, *S. flexneri*, *S. boydi* and *S. sonnei*. The purpose of this study was to determine plasmid profiles, antibiotic resistance and phenotypic virulent by Congo red between *S. flexneri* strains.

Methods The isolated bacteria were identified with standard bacterial and biochemical methods. Plasmids were isolated by alkaline lysis method. Antibiotic susceptibility test was performed according to 'Kirby-Bauer' method. Serological reactions were carried out by slide agglutination tests with both polyclonal and monoclonal antiserum kits. Virulent strains were isolated on a TSA plate + 0.003% Congo red dye concentration. *S. flexneri* was isolated from faecal specimens of children attending the three children's hospitals.

Results From 350 isolated *Shigella* species, 142 (40/57%) were *S. flexneri*. Of patients 40/7% were female and 59/3% were male. Analysis of plasmid bands revealed that all of them contained multiple plasmid bands (1–5 plasmid bands). A total of 11 distinct plasmid profile patterns were identified. Of *S. flexneri* isolates, 75/30% were resistant to trimethoprim-

sulfometoxazol, 91/3% to ampicillin, 95% to tetracycline, 70/3% to cephalexin. All of the isolates were sensitive to ciprofloxacin. Our results showed that 38/9% were serotype 2. In this study, 45/56% of *S. flexneri* were Congo red-positive and all of them (100%) were haemolysin-positive on blood agar plates.

Conclusion *S. flexneri* isolation is important, because *S. flexneri* is the prevalent strain in developing countries, including Iran, and health care notes may prevent shigellosis. Antibiotic-resistant determination in each case may prevent drug resistance increase. Since Congo red binding test is cheap and simple it can be used to determine virulence properties of *S. flexneri*.

JSM 32 ESBL type SHV-12-producing *Enterobacteriaceae* isolates from children with bacteraemia in Tanzania – involvement of plasmids

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During a study on paediatric bloodstream infections at a tertiary hospital in Tanzania blood cultures were collected from August 2001 to August 2002. A high proportion (19%) of the *Enterobacteriaceae* isolates recovered from the blood cultures had an ESBL phenotype and were resistant to most of the locally available drugs. The presence of SHV-, TEM- and CTX-M genotypes was detected and identified by PCR and sequencing. The genetic relatedness of the isolates was evaluated by Amplified Fragment Length Polymorphism (AFLP). Conjugation experiments were done by a filter mating method. Plasmids were isolated and detected by an alkaline lysis protocol and S1-PFGE. The SHV-12, SHV-2a, TEM-63 and CTX-M-15 ESBL genotypes were identified. Four of the 9 ESBL producing *Klebsiella pneumoniae* isolates had the SHV-12 genotype. The *bla*SHV-12 gene was located on large plasmids which were successfully transferred to *E. coli* by conjugation. Genes coding for resistance to aminoglycosides were co-transferred. Phenotypic resistance to chloramphenicol, trimethoprim-sulfamethoxazole and doxycycline was also co-transferred. The one *Salmonella enterica* serotype Newport isolate, five *E. cloacae* isolates and two *Pantoea* spp. isolates which were ESBL-positive also harboured the *bla*SHV-12 gene. All isolates were obtained throughout the study period and from five different clinical wards. None of the four SHV-12 positive *K. pneumoniae* isolates were related as determined by AFLP, but three of them harboured plasmids with a similar restriction pattern. Two of the *E. cloacae* isolates were related. All isolates harboured at least one plasmid larger than 100 kb. None of the 9 ESBL-positive *E. coli* isolates collected during the study period had the *bla*SHV-12 gene, but a SHV-12-positive *E. coli* isolate from a urine sample from another department at the hospital was collected a few months later. A high proportion (46%) of the ESBL producing blood culture isolates collected during the 1-year study period had the SHV-12 genotype. Few isolates were related as determined by AFLP, but some of the unrelated *K. pneumoniae* isolates had related conjugative plasmids. This suggests the possibility that spread of resistance genes is plasmid mediated and not due to dissemination of clonally or related bacterial strains.

Authors

Posters – Late Abstracts

JSM 33 Contribution of quorum sensing molecules to the virulence of *Pseudomonas aeruginosa* in an experimental urinary tract infection model

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Quorum sensing signals have been proposed to play most important role in the pathogenesis of respiratory tract and burn wound infections caused by *Pseudomonas aeruginosa*. This pathogen has been reported to monitor its cell density as well as expression of virulence determinants by quorum sensing signal mechanisms operative through autoinducers. In the present investigation we have studied the contribution of quorum sensing signals during course of *P. aeruginosa* induced urinary tract infection (UTI). Fifty uroisolates and one standard strain of *P. aeruginosa* were initially screened for the production of quorum sensing signals both qualitatively and quantitatively. For further studies, quorum-sensing-positive and quorum-sensing-negative strains (2) of *P. aeruginosa* were checked for their virulence in an acute ascending UTI mouse model. It was observed that quorum deficient strain was significantly less virulent as compared to quorum sensing producer strain during the course of infection. The present study brings out that quorum sensing signals are important for the pathogenesis of urinary tract infections caused by *P. aeruginosa*.

JSM 34 Alteration in virulence traits of planktonic and biofilm cells of *Pseudomonas aeruginosa* following exposure to chlorhexidine and iodophor

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Urinary tract infections (UTIs) are most common bacterial infections affecting human throughout their life span. Urinary tract can be uncomplicated or complicated. *Pseudomonas aeruginosa* is the third most common pathogen accounting for 35% cases of catheter associated nosocomial UTIs. It has a tendency to form biofilms on surface of indwelling catheters. Biofilms are resistant to host defense mechanisms as well as to antimicrobial agents hence are of major concern for treating clinicians. Efforts have been done to prevent catheter-induced UTIs using different strategies like coating of catheter surface with silver compounds but only limited success has been achieved. The present investigation was planned to study the effect of chlorhexidine and iodophor on elaboration of virulence factors like alginate, siderophore, hemolysin, hydrophobicity and protease by planktonic and biofilm cells of *P. aeruginosa*. Significant decrease in elaboration of these virulence traits by planktonic and biofilm cells was observed following growth of *P. aeruginosa* in chlorhexidine and iodophor. The present study brings out the efficacy of these disinfectants against *P. aeruginosa*. Decreased elaboration of virulence traits will help in preventing lodgment of bacteria and controlling infections induced by *P. aeruginosa*. Implications of these findings in combating infections will be discussed.