Emerging Technologies of Medical Importance for the Diagnosis of Infectious Diseases and the Detection of Pathogenic Microbes

organized in collaboration with the Chinese Medical Association & Peking Union Medical College Hospital
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ASM CONFERENCES MISSION

To identify emerging or underrepresented topics of broad scientific significance.

To facilitate interactive exchange in meetings of 100 to 700 people.

To encourage student and postdoctoral participation.

To recruit individuals in disciplines not already involved in ASM to ASM membership.

To foster interdisciplinary and international exchange and collaboration with other scientific organizations.

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Centers for Disease Control and Prevention

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ACKNOWLEDGMENTS

This conference is organized in collaboration with the Chinese Medical Association (CMA) and the Peking Union Medical College Hospital (PUMCH). ASM gratefully acknowledges their assistance in making this conference possible.

The Organizers, ASM, CMA, and PUMCH would like to acknowledge the following for their generous financial support of this conference:

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BD Diagnostics
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GENERAL INFORMATION

HOTEL ADDRESS
All conference activities are held at the Beijing International Hotel, No. 1 Jianguomenwai Avenue, Beijing, 10004, China.

GENERAL SESSIONS
All general sessions will be held in the Grand Ballroom at the Beijing International Hotel. A name badge is required for entry into all sessions. In consideration of other participants, no children are permitted in the sessions.

POSTER SESSIONS
Poster boards are located in the Sunshine Room at the Beijing International Hotel. Presenters should mount posters on the board space bearing the number assigned to the abstract and stand by the poster during the assigned poster session (A, B, C, or D) as noted in the program schedule. Each poster is allotted one board face. Please check your assigned number in the abstract index and mount your poster on the board space bearing that number.

Official poster sessions will be held on April 7, 8, and 9 as noted within the program. Please stand at your poster during your assigned session.

SOCIAL EVENTS

Welcome Reception, Sunday, April 6, 2008, 6:45 - 8:00 pm, in the Sunshine Room
Included in conference registration fee. Guest tickets are available from the registration staff for $50 USD, or RMB 350.

Conference Closing Dinner, Wednesday, April 9, 2008, 8:00 - 10:00 pm, in the Grand Ballroom
Included in conference registration fee. Guest tickets are available from the registration staff for $90 USD, or RMB 650.

Group Tour to the Great Wall, Thursday, April 10, 2008
Not included in conference registration fee. Tickets are available from the CYTS travel agent desk in the registration area for RMB 350. Tour subject to cancellation should registration not meet minimum required number of participants.

STUDENT TRAVEL GRANTS
ASM encourages the participation of graduate students and new postdocs at ASM Conferences.
To support the cost of attending the conference, ASM has awarded travel grants of $500 to each of the following individuals:

Mansur-ud Ahmad
Chiao-Shan Chen
Jiabin Dong
Francesca Donnarumma
Jhabindra Ghimire
Siyu Hu
Fupin Hu
Yong Huang
Tayyaba Ijaz
Yang Liu
Zanzan Liu
Xin Ma
Luan Mingchun
Qin Pan
Muhammad Shahzad
Tatsuya Unno
Ina Willemesen
Xiaohu Xia
Daojun Yu
Yi Zhang
Yumei Zhu
Emerging Technologies of Medical Importance

APRIL 6, 2008

4:00 – 6:45 pm  **Session 1: Opening Session**
Welcome and Goals of the Meeting  
*Lance Peterson and Minjun Chen*

Keynote Addresses:

Designing Artificially Intelligent Tools for use with Medical Databases  
*Daisy Wong*

Detection of XDR-TB  
*Barry Kreiswirth*

Rapid Detection of Resistance  
*Hui Wang*

Collaboration on Global Infection Threats  
*Fred Tenover*

6:45 – 8:00 pm  Opening Reception

APRIL 7, 2008

8:30 – 10:45 am  **Session 2: Applications of Molecular Biology to Non-Viral Microbial Nucleotide Target Detection**  
Chair - David Durack

Real-time PCR and Related Technologies  
*Paul Savelkoul*

Assessment of FDA/Agency-Cleared Diagnostics  
*Lance Peterson*

Developing Novel In-House Technologies  
*Karen Kaul*

Coffee Break

11:00am – 12:00 pm  **Poster Session A**

12:00 – 1:30 pm  Lunch with the Posters

1:30 – 3:45 pm  **Session 3: Applications of Novel Technologies to Infectious Diseases**  
Chair - David Persing

Detection of MDR Gram-Negative Bacilli  
*Abhay Chowdhary*
SCIENTIFIC PROGRAM

Detection of Sepsis - The SeptiFast test, Rapid Detection of Bacterial and Fungal DNA in Bloodstream Infections

*Lyn Waring*

Detection of Host Response Targets for Diarrhea

*J-P Jin*

Coffee Break

4:00 - 5:00 pm  **Poster Session B**

5:30 – 7:00 pm  Traditional Food and Drink Reception and General Individual Discussions (posters remain open for discussion)

Dinner on your own

APRIL 8, 2008

8:30 – 10:45 am  **Session 4: Applications of Novel Systems for Detection of Unexpected or Unwanted Events – Integrating the Laboratory and Infection Control**

Chair - Tom Riley

Bacterial Typing and Resistance Surveillance Programs

*Geoffrey Coombs*

Application of GIS for Analyzing the Risk and Spread of Infectious Diseases

*Hiroshi Suzuki*

Using Novel Sources for Microbial/Infection Data Information in Healthcare

*Ari Robicsek*

Coffee Break

11:00 am – 12:00 pm  **Poster Session C**

12:00 – 1:30 pm  Lunch with the posters

1:30 – 5:00 pm  **Poster Slide Session**

Individual 15-minute oral presentations selected from the submitted abstracts (Presenter is noted in Bold)

*Molecular Analysis of the Causes of Severe Acute Respiratory Illness*

*K. C. Sumino, N. S. Kolovos, M. Q. Arens, R. S. Buller, D. J. Hormozdi, A. M. Gaynor, E. Agapov, D. Wang, G. A. Storch; Washington University School of Medicine, St. Louis, MO*

*Simultaneous Detection of Severe Acute Respiratory Syndrome Coronavirus (SARS CoV) and Influenza viruses A using Real-Time PCR*

*W. C. Yam, W. H. Seto, P. L. Ho, K. H. Chan, W. T. Hut; ‘University of Hong Kong, Hong Kong,, CHINA, ‘Queen Mary Hospital, Hong Kong,, CHINA, ‘Princess Margaret Hospital, Hong Kong,, CHINA*
Multiplex Real-Time PCR Detection of Three Drug-Resistant Mutations in Mycobacterium tuberculosis in a Single Tube with Displacing Probes
H. Wen\textsuperscript{1}, S. Hu\textsuperscript{1}, J. Niu\textsuperscript{2}, J. Huang\textsuperscript{1}, Y. Zhuang\textsuperscript{1}, Q. Li\textsuperscript{1}; \textsuperscript{1}Department of Biomedical Sciences, School of Life Sciences, Xiamen University, Xiamen361005, CHINA, \textsuperscript{2}Xiamen Center for Disease Control and Prevention, Xiamen361021, CHINA

Pyrosequencing\textsuperscript{TM} analysis of the gyrB gene to differentiate bacteria responsible for diarrheal diseases
X. Hou, Q. Cao, H. Jia, Z. Chen; Institute of Infectious Diseases, First Affiliated Hospital, Medical College, Zhejiang University, Hangzhou, CHINA

Multi-drug Resistance Profiles Correlate with Genetic Profiles in Clinical Isolates of Acinetobacter baumannii
H. H. Xu, S. C. Valentine; California State University, Los Angeles, Los Angeles, CA

Rapid genotyping of CTX-M extended-spectrum beta-lactamases by denaturing high-performance liquid chromatography
L. Xu\textsuperscript{1}, J. Evans\textsuperscript{1}, T. Ling\textsuperscript{2}, K. Nye\textsuperscript{1}, P. Hawkey\textsuperscript{1}; \textsuperscript{1}HPA, Birmingham, UNITED KINGDOM, \textsuperscript{2}Prince of Wales Hospital, Hong Kong, CHINA

Coffee Break

Information Technology Applications for Infectious Diseases and Pathogenic Microbes: A Unified Multidisciplinary Approach
Y. E. Lin\textsuperscript{1}, V. L. Yu\textsuperscript{1}; \textsuperscript{1}National Kaohsiung Normal University, Kaohsiung, TAIWAN, \textsuperscript{2}University of Pittsburgh, Pittsburgh, PA

Investigation of Increased Healthcare-Associated Urinary Tract Infections (UTI) Identified by Automated Surveillance
D. M. Hacek, A. Fisher, A. Ogle, L. Kasparian, A. Robicsek, L. R. Peterson; Evanston Northwestern Healthcare, Evanston, IL

Rigorous MRSA Surveillance Using RT-PCR: 22-Month Experience
P. Tsang, T. Chan, K. Holland, J. Murillo; Newark Beth Israel Medical Center, Newark, NJ

Epidemiological Profile of Nosocomial Infections in a Public sector Tertiary Care Hospital of Pakistan
T. Ijaz\textsuperscript{1}, F. A. Ranjha\textsuperscript{1}, M. A. Munee\textsuperscript{1}, A. R. Khawaja\textsuperscript{1}, M. Imran\textsuperscript{1}, M. A. Khan\textsuperscript{1}, M. K. Shahzad; Mayo Hospital, Lahore, PAKISTAN; University of Veterinary and Animal Sciences, Lahore, PAKISTAN

Importance of a Positive Stool-PCR Test in Detection of Helicobacter pylori Infection in Children
T. Falsafi\textsuperscript{1}, R. Favaedi\textsuperscript{1}, F. Mahjoub\textsuperscript{2}; \textsuperscript{1}Azazra University, Tehran, IRAN (ISLAMIC REPUBLIC OF), \textsuperscript{2}Medical Center for Children, TEHRAN, IRAN (ISLAMIC REPUBLIC OF)

Simultaneous Detection of Antibodies to Hepatitis C Virus Proteins Using the Luminex System to Distinguish Acute from Chronic HCV Infection
A. Araujo\textsuperscript{1}, I. Astrakhantseva\textsuperscript{1}, Z. Dimitrova\textsuperscript{1}, T. Ulanova\textsuperscript{2}, A. Obriadina\textsuperscript{2}, H. Fields\textsuperscript{1}; \textsuperscript{1}Centers for Disease Control and Prevention, D VH, Atlanta, GA, \textsuperscript{2}RPC Diagnostic Systems, Nizhny Novgorod, RUSSIAN FEDERATION

Dinner on your own
APRIL 9, 2008

8:30 – 10:00 am  
Session 5: Applications of Integrated Systems for Management and Detection of Unexpected or Unwanted Events

Monitoring the Geographic Spread of Antimicrobial Resistant Bacteria  
*Hajo Grundman*

Control of Antimicrobial Resistance: An Integrated Approach  
*Jan Kluytmans*

Coffee Break

10:15 – 11:15 am  
Poster Session D

11:30 am – 1:00 pm  
Lunch with the posters

1:00pm – 3:45 pm  
Session 6: Implementation and Outcome Monitoring of Novel Healthcare Applications for Microbiology and Infectious Diseases

Developing a Plan for Introducing a New Program  
*Lance Peterson*

The Role of Mathematical Models  
*Marjan Wassenberg*

Emerging Technology - Critical Factors for Application in Routine Point of Care Testing  
*Gary Thorpe*

Coffee Break

3:30 – 6:30 pm  
Session 7: Application of Emerging Technologies

Molecular Diagnosis of Fungal Diseases  
*Tsung-Chain Chang*

*Staphylococcus Epidermidis*: From Genomics to Clinical Isolates  
*Yumei Wen*

Analysis of Microarray Data for Host/Pathogen Interaction  
*Jody Lindsay*

Rapid and On-Demand Detection and Characterization of Staphylococci Causing Bloodstream Infections  
*Yi-Wei Tang*

8:00 – 10:00 pm  
Congress Dinner

APRIL 10, 2008

All day – Group Visit to the Great Wall of China  
register at the CYTS tour desk - additional fee required
Manipulation of Nuclear Processes by DNA Viruses

S1:1

Designing artificially intelligent tools for use with medical databases

D. Wong; Cardinal Health/MedMined™ Services, Birmingham, AL, USA

Infection control is a quality control activity concerned with the detection, quantification and prevention of nosocomial infections (NIs). Early recognition of NIs is critical for the control of these events, and requires proactive surveillance as part of hospital infection control programs. Such surveillance is intensely resource consumptive. Timely detection of patterns of infections and drug-resistance requires extensive analyses of large volumes of laboratory data as well as patients’ spatial and temporal history data. The Data Mining Surveillance System (DMSS) developed by clinical scientists at Cardinal Health has been successful in both uncovering new patterns of interest and detecting known outbreaks in hospital-wide historical data continuously [1]. DMSS transformed the practice of infection control from the tradition of manually collecting, collating and analyzing data to one of technology driven process improvement, resulting in infection prevention and improving patient safety.

Data mining uses artificial intelligence techniques to analyze large volumes of data to identify critical and previously unsuspected patterns. A range of artificial intelligence and statistical techniques are available. DMSS uses association rules with statistical analyses to identify patterns of changes in laboratory and patient movement data, and uses visualization to report the patterns to end users. This paper will discuss the application of association rules for early detection of change in infection trends and antimicrobial resistance. Both the benefits and challenges of utilizing artificial intelligence techniques to very large databases will be discussed.

Successful data mining requires usable data. For DMSS, the data sources are laboratory information system (LIS), the admit-discharge-transfer (ADT) system, and the hospital census system. Clinical laboratory data, especially clinical microbiology data, are poorly structured and contain free text and natural language. The data must be cleaned, transformed, and mapped to standard lexicon prior to selection for data mining. The data and content structure may change due to LIS upgrades, introduction of new tests and discontinuation of old ones. For these reasons, in an operational data mining system, the data sources must be actively monitored for changes so that the processing is adjusted in a timely manner.

The selection and interpretation of the patterns from data mining must be guided by domain expertise. Otherwise, end users could suffer from pattern glut and the knowledge discovered may end up unused. DMSS supports expert pattern selection and interpretation.

For future developments, access to additional electronic data could extend the capability of DMSS. The vision of a system that encompasses the collective knowledge of a variety of domain experts to guide data mining and interpretation on a continual basis will also be discussed.

S1:3

Rapid detection of resistance

H. Wang; Peking Union Medical College Hospital, Beijing, CHINA

In the past 10 years, many new molecular-based methods had been applied in the rapid detection and reporting of antimicrobial-resistant pathogens. New PCR-based methods such as multiplex PCR and real-time PCR had been used in this field. For example, a real-time PCR assay for identification of MRSA isolates containing PVL was reported in 2005. This PVL assay was used in a triplex format allowing simultaneous amplification of mecA, nuc, and pvl genes. In 2006, a real-time duplex PCR assay for the simultaneous detection of Enterococcus faecium and vanB resistance genotype was developed. Commercial kits to detect MRSA have been available. The BD GeneOhm MRSA real-time PCR assay, formerly called the IDI-MRSA assay, offers rapid identification of MRSA-colonized patients in as little as 2h, with a sensitivity of 92.5% and a specificity of 96.4%. In 2008, it is reported that the determination of MRSA directly from positive blood cultures is achieved by Isothermal Amplification and Disposable Detection Device. There has also been great progress in the rapid diagnosis of resistance in Gram-negative bacteria. A real-time PCR protocol for rapid detection of the most common bla TEM in a single reaction was reported in 2007. Also in 2007, a multiplex, real-time PCR assay using SYBR Green I and the Roche LightCycler was developed. The assay can rapidly identify eight genes encoding tetracycline resistance efflux pumps including tet (A) to (J). A novel, multiplex, real-time TaqMan PCR assay to detect and type bla TEM genes was described in 2007 which is an improvement on previously described techniques with respect to reduced assay time, elimination of the need for protracted post-PCR processing and the convenience of a single reaction vessel.

Several microarray methods had been tried in Gram-negative. In 2007, a microarray-based assay was developed and applied, which provides a rapid, sensitive and specific detection of fluoroquinolone-resistant E. coli in urine. A miniaturised microarray for the detection of antimicrobial resistance genes in Gram-negative bacteria was reported in 2008, in which genes encoding resistance to aminoglycosides, trimethoprim, sulfonamides, tetracyclines and beta-lactams as well as extended-spectrum beta-lactamases were included. The importance of rapid detection of resistance in pathogens relies not only in that effective diagnosis and treatment of infection depend mainly on the performance of the laboratory to detect emerging resistant pathogens accurately, but also in that cost-effectiveness is paid more attention in nowadays clinical practice.

It is encouraging to witness the great advance in this field and undoubtedly, there will be more and more creative methods in the future.
S1:4
Collaboration on Global Infection Threats
F. N. Tenover; Centers for Disease Control and Prevention, Atlanta, GA, USA

The spread of infectious diseases globally continues to be a major public health concern. Significant collaborations have been formed among the World Health Organization, the Center for Disease Control and Prevention in the United States, the Chinese Center for Disease Control and Prevention, and multiple other international agencies. The International Committee of the American Society for Microbiology (ASM), in collaboration with these agencies, continues to foster both educational and laboratory programs to strengthen disease detection and reporting. On example of ASM’s involvement is the recent training of technicians to process specimens for detection of Mycobacterium tuberculosis in Namibia. Another significant advance is the World Health Organization's Global Outbreak Alert and Response Network (GOARN). The major goals of this network are to “help countries with disease control efforts by providing rapid and appropriate technical support to affected populations”, “to investigate and characterize events and assess risks of rapidly emerging epidemic disease threats”, and to “support national outbreak preparedness by ensuring that responses contribute to sustained containment of epidemic threats”. In the last 8 years, over 400 experts have contributed their skills through GOARN in response to over 50 events worldwide. Through the development and strengthening of global cooperative networks, the devastating impact of emerging infectious diseases can be often be reduced.

S2:2
Assessment of FDA/Agency-Cleared Diagnostics
L. R. Peterson; Evanston Northwestern Healthcare, Evanston, IL

During recent years the United States Food and Drug Administration have cleared several new molecular diagnostic tests for use in clinical laboratories. When contemplating introduction of novel technology into diagnostic testing there are many facets of use that need to be considered. The performance characteristics of the test (e.g., sensitivity, specificity, and predictive values) are critical. Also important is the application of the test (e.g., surveillance versus infection diagnosis), what population(s) will be eligible for testing, the cost/benefit of the new test, and how will the results be interpreted. PCR-based amplification tests in this category include those for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Roche), group B streptococci (BD GeneOhm, Cepheid), MRSA (BD GeneOhm, Cepheid), *S. aureus* (MSSA) and MRSA (BD GeneOhm), enterovirus (Cepheid), and respiratory virus panels (Lunimex, Prodesse).

The purpose of these tests range from surveillance (MRSA and group B streptococci) to the detection of specific infection by assaying direct patient specimens. Decisions based on introducing any new test (including novel molecular testing) should be based on the following criteria:

1. Is there a need for a new test - why do this?
   a. Do we perform the testing now?

2. Are the new test performance characteristics satisfactory?
   a. Sensitivity, specificity and predictive values (with our disease prevalence) compared to a ‘gold standard’ or the current test
   b. Testing turn around time and documented need for rapid diagnosis
   c. Resources - capacity of technical persons to do the testing
   i. Do we have the skill?
   ii. Do we have the hands-on time?
   iii. Is new equipment or remodeling required?

3. Will the new test improve patient care?
   a. Detect more patients with infection
   b. Improve antibiotic prescribing
   c. Reduce disease transmission for infection control

The purpose of this presentation is to outline a guide for assessing the need for introducing a new molecular diagnostic test into the laboratory and present the performance characteristics of these various assays, review how they are reported to perform when applied to their intended use, and discuss their potential benefit in the prevention and treatment of human disease.

S3:1
Detection of Multidrug resistant Gram negative bacilli (MDR GNB)
A. Chowdhary; Grant Medical College & Sir J J Hospital, Mumbai, INDIA

The antimicrobial resistance of GNB is showing progressive increase during last few decades, leading to increased incidence of outbreaks of infection due to presence of MDR bacteria in various populations in hospital and community settings. Epidemiologically important MDR GNBs are ESBL producing organisms due to resistance to first line therapies, unusual agents with unusual patterns of resistance within a facility e.g. The first isolates of *Burkholderia cepacia* or *Raltoria spp.* in non CF patients or a quinolone resistant strains of *Pseudomonas* in a facility and difficult to treat because of innate or acquired resistance to multiple classes of antimicrobial agents eg. *Stenotrophomonas maltophilia*, *Acinetobacter baumannii* etc. In routine Clinical diagnostic laboratories, classical biochemical tests and a combination of intuition and stepwise analysis of the results are used to identify the isolates. Genotypic, Phenotypic and Phylogenetic techniques can be used to identify bacteria. Genotypic methods give information on nucleic acid (DNA and RNA) present in the cell. The chemotaxonomic methods,
Emerging Technologies of Medical Importance

adjunct to traditional methods for the early diagnosis of sepsis, detected. Overall, this test has the potential to be a valuable tool, as the test was not designed to detect and in no cases were these exclusivity were performed on 31 common clinical isolates that were representative of 1,548 clinical isolates originating from various geographic areas in Europe (northern, central and southern Europe) and the United States, resulting in an overall specificity of 98.8%. Comparative analysis of the analytical specificity showed that the SeptiFast test was developed in response to the need for faster diagnostic tools for patients with sepsis. It is a multiplex real-time PCR-based assay for the rapid detection and identification of 25 clinically important pathogens directly from whole blood in less than 6 hours. To increase the sensitivity of the test and to minimize potential contamination coming from reagents or consumables, this assay utilizes high-quality DNA free reagents and plastic ware. This presentation will describe the assay and the tests done to establish its analytical precision, sensitivity and specificity. This included tests for inclusivity on 1,548 clinical isolates originating from various geographic areas in Europe (northern, central and southern Europe) and the United States, resulting in an overall specificity of 98.8% compared to conventional microbiology. The analytical specificity in 102 healthy blood donors was 100%. In addition, tests for exclusivity were performed on 31 common clinical isolates that the test was not designed to detect and in no cases were these detected. Overall, this test has the potential to be a valuable adjunct to traditional methods for the early diagnosis of sepsis, without the need for prior incubation of cultures.

S3:2

The SeptiFast Test, Rapid Detection of bacterial and fungal DNA in bloodstream infections

L. Waring; Roche Molecular Systems Inc., Pleasanton, CA, USA

The SeptiFast test was developed in response to the need for faster diagnostic tools for patients with sepsis. It is a multiplex real-time PCR-based assay for the rapid detection and identification of 25 clinically important pathogens directly from whole blood in less than 6 hours. To increase the sensitivity of the test and to minimize potential contamination coming from reagents or consumables, this assay utilizes high-quality DNA free reagents and plastic ware. This presentation will describe the assay and the tests done to establish its analytical precision, sensitivity and specificity. This included tests for inclusivity on 1,548 clinical isolates originating from various geographic areas in Europe (northern, central and southern Europe) and the United States, resulting in an overall specificity of 98.8% compared to conventional microbiology. The analytical specificity in 102 healthy blood donors was 100%. In addition, tests for exclusivity were performed on 31 common clinical isolates that the test was not designed to detect and in no cases were these detected. Overall, this test has the potential to be a valuable adjunct to traditional methods for the early diagnosis of sepsis, without the need for prior incubation of cultures.

S3:3

Detection of host response targets for diarrhea

J.-P. Jin and Lance Peterson; Evanston Northwestern Healthcare and Northwestern University Feinberg School of Medicine, 2650 Ridge Ave., Evanston, IL 60201, USA

Clostridium difficile-associated diarrhea (CDAD) has become a crucial pathogen with new virulent strains emerging, especially in hospital infections. The standard method for the diagnosis of CDAD relies on the detection of C. difficile toxin A, B, or A and B. However, a positive result for C. difficile toxins in diarrhea stool samples does not reliably indicate a causal relationship to the disease. Therefore, additional tests that are directly correlated to the specific pathology of CDAD need to be developed. C. difficile toxin B is the unique toxin always present in CDAD. The pathogenic action of toxin B involves disruption of the actin cytoskeleton of the colonic epithelial cells. Cell adhesion is dependent on the function of actin cytoskeleton and the effects of C. difficile toxin B would result in accelerated dissociation of colonic epithelial cells. This unique cellular pathology suggests a target for the development of an assay to measure the effects of C. difficile toxin B on human host colonic epithelial tissue. To test a hypothesis that an increase in colonic epithelial cytoskeleton proteins in the patient stool would indicate a host response to C. difficile infection and, therefore, can be used as a diagnostic target, we have demonstrated an increased release of tropomyosin, a major component of the actin cytoskeleton, in C. difficile patient stool samples. Based on the established biochemical features of tropomyosin, an assay protocol that can be readily performed in clinical laboratories has been developed. This approach has also resulted in the development of a protein-based bio-bar code assay. The demonstration of colonic epithelial cellular response as an indicator of the specific host response to CDAD presents a novel example of the translation of biochemistry and cell biology knowledge into the improvement of clinical diagnosis of infectious diseases.

S4:1

Bacterial Typing and Monitoring Resistance Surveillance Programs

G. Coombs; Royal Perth Hospital, Perth, Western Australia, AUSTRALIA

Infections caused by antibiotic resistant bacteria are an established problem for patients being treated in hospitals and increasingly in the community setting. Resistance surveillance is one of the main elements in any strategy to control antimicrobial resistance. In the microbiology laboratory two basic types of surveillance are possible: passive (data on susceptibilities and resistance are collated from those generated by the routine susceptibility testing of clinical isolates) and targeted (specific species or groups of species are examined in greater detail eg broader range of antimicrobials, MIC distribution data, molecular clone typing, molecular tests for mechanisms of resistance). National laboratory-based surveillance is a key part of the antimicrobial resistance management plan of many countries and for the World Health Organisation in its
Global Strategy for Containment of Antimicrobial Resistance. In Australia targeted national surveillance has been conducted by the Australian Group on Antimicrobial Resistance (AGAR) [http://www.antimicrobial-resistance.com] for over 20 years. AGAR participants include clinical microbiologists and laboratory scientists from 32 teaching hospitals and pathology service providers located throughout Australia. The group conducts surveys on the administrative resistance patterns of Staphylococcus aureus, Escherichia coli, Klebsiella spp, Enterobacter spp, Streptococcus pneumoniae and Enterococcus spp biennially and Haemophilus influenzae periodically. Using standardised methodology AGAR has been able to collect ongoing susceptibility data on what is happening in Australia over long periods of time.

This has led to important benefits within Australia including the ability to allow more rational use of antibiotics based on known Australia wide resistance patterns. Bacterial epidemiological typing also assists in controlling antimicrobial resistance.

In Western Australia (WA), a statewide MRSA management policy introduced in 1992 has prevented MRSA from becoming established in WA acute-care hospitals. A key element of this policy involves the epidemiological typing of all MRSA by a central typing laboratory. Using SCCmec and multilocus sequence typing MRSA are characterised as epidemic MRSA (EMRSA) or non EMRSA. Although imported EMRSA still occasionally caused single-strain outbreaks in hospitals rapid clone identification and infection control interventions have contained them. Imported Panton-Valentine leucocidin (PVL) positive MRSA clones, including USA300, have recently been isolated in the WA community. The emergence of these strains is a major public health concern and emphasizes the importance of epidemiological typing in tracing the origin of isolates and designing intervention strategies and antimicrobial drug prescribing policies for their control, if possible, in the community.

S4:2

Application of GIS in Outbreak Investigation Involving Infectious Diseases

H. Suzuki: Niigata University, Graduate School of Medical and Dental Sciences, Niigata, JAPAN

John Snow’s classic investigation of cholera represents one of the earliest and well-known successful uses of mapping both disease and factors possible affecting disease, in this case, source of water. Geographic information system (GIS) provides new sophistication to an old concept. GIS can be used to map a wide array of variable including location, disease occurrence, and environment, and can provide a valuable analysis tool for epidemiology and public health personnel. The principal advantage of GIS is its spatial analysis capabilities. The mode of transmission is a critical factor in any GIS assessment of non-vectorized diseases. We present here the special analysis of cholera outbreak in Zambia and seasonal influenza outbreaks in Japan by using GIS.

1. Cholera outbreaks in Zambia

We did special analysis of risk factor of cholera outbreak in Lusaka, Zambia by using GIS. Plotting of the cholera patients indicated that almost all patients were adults in the first stage of the outbreak, while the numbers of affected infants and young children under 5 years old increased gradually as the epidemic continued. Cholopleth map of cholera cases demonstrated that higher incidence rate of cholera in administrative boundary was statistically associated with lower coverage of latrines and effective drainage systems.

2. Influenza infections in Japan.

In order to know the spatio-temporal dynamics of influenza in Japan, we used a GIS with several kinds of influenza surveillance information. GIS map showed clearly the spreading mode of peak influenza activity throughout Japan in each epidemic, and predicted that epidemic in the pandemic period run through the whole country quite rapidly, probably less than 3 weeks. Furthermore, basing on data on school-based influenza surveillance during inter-pandemic period, influenza outbreaks spread widely in flat areas, and along traffic routes in mountain areas. Study on spatial distribution of influenza A and B virus infections showed that influenza A virus infections spread more widely than influenza B virus infections. Thus, those observations suggest that GIS be quite valuable for strengthening the whole process of epidemiological surveillance information management and analyses.


PS:1

Molecular Analysis of the Causes of Severe Acute Respiratory Illness

K. C. Sumino, N. S. Kolovos, M. Q. Arens, R. S. Buller, D. J. Hormozdi, A. M. Gaynor, E. Agapov, D. Wang, G. A. Storch; Washington University School of Medicine, St. Louis, MO

We are using molecular methods to study viral causes of severe acute respiratory illness (SARI). For this study, we have collected 485 respiratory samples from patients in the intensive care units of the hospitals of the Washington University Medical Center in St. Louis, Missouri. 17% of samples are from children. All adult samples were bronchoalveolar lavage (BAL); samples from children included BAL and tracheal aspirates. Most specimens (79%) were analyzed by conventional FA staining and culture. All specimens were tested using the Eragen Multicode PLx Respiratory Virus Panel, a commercial highly multiplexed PCR assay with a Luminex-based detection system that detects 17 respiratory viruses. Specimens were also tested by PCR for human bocavirus and the WU and KI respiratory polyomaviruses. Finally, we performed mass sequencing (384 sequences per sample) on 20 BALs that were negative for viruses by any method. One or more respiratory viruses were detected in samples from 79 patients including 13% of adults and 35% of children (P<.001). Viruses detected included rhinovirus (n=32), RSV (n=14), HPIV (n=12), Bocavirus (n=6), Flu A (n=6), coronaviruses (n=4), KI (n=4), Adeno (n=3), Flu B (n=2), HMPV (n=2). Rhinoviruses included viruses from groups A and B as well as viruses corresponding to newly described rhinoviruses. Of the specimens analyzed by FA and culture, use of molecular techniques increased the yield of respiratory virus detection by 3-fold. Mass sequencing of the 20 virus-negative samples did not reveal evidence of any viruses. However, one sample yielded multiple sequences matching Pneumocystis jiroveci (which had been identified by conventional testing) and one sample yielded sequences most homologous with Cryptococcus neoformans. No fungus was detected in this patient during life. Clinical features were analyzed in 265 adult
patients of whom 55% were immunocompromised. Patients with and without viruses detected did not differ with respect to age, gender, percent immunocompromised, length of ICU stay or hospitalization, days of mechanical ventilation or oxygen therapy, or days of antibiotic therapy. For pediatric patients, days of mechanical ventilation were similar in those with and without respiratory viruses, but those with viruses received fewer days of antibiotic therapy (8.7 vs 18.8, P = .08). We conclude that molecular testing dramatically increases the yield of respiratory virus detection in patients with SARI. The clinical significance of these viruses is still under investigation. Mass sequencing may reveal pathogens not detected by conventional or PCR-based molecular methods.

PS:2

Simultaneous Detection of Severe Acute Respiratory Syndrome Coronavirus (SARS CoV) and Influenza viruses A using Real-Time PCR

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A total of 1,150 nasopharyngeal aspirates were collected from patients suffering respiratory tract infections or flu syndrome between 2003 and 2007. Conventional laboratory diagnosis for influenza virus A and B, parainfluenza virus types 1, 2, and 3, respiratory syncytial virus RSV and adenovirus were performed using immuno-fluorescence / commercial screening kit and viral culture on MDCK, HepII and LLCMK2 cells. Patient serum samples were also collected for serologic testing towards SARS CoV. A multiplex real time PCR was designed against SARS CoV (P-gene) and Influenza A (M-gene) with confirmation by specific fluorescent labeled hybridization probes. Among the 1,150 samples, 323 were confirmed positive for SARS CoV among patients in 2003 SARS epidemic and SARS CoV was not detected for samples between 2004-2007. Influenza A viruses (H1 and H3 subtypes) were detected among 275 samples and no H5 subtype was identified. The resolved performance of real time PCR was validated against conventional laboratory diagnosis with 86% and 93% sensitivity for SARS CoV and Influenza A viruses respectively. The real time PCR assay exhibited a 100% specificity for the detection of both viral pathogens. Since the first epidemic of SARS CoV in 2003 overlapped with flu seasons in Hong Kong, laboratory diagnosis relied on separate PCR assays on each viral pathogen which was tedious and laborious. Our real time PCR assay provides rapid differential diagnosis of both viral pathogens in a single reaction, saving 30% manpower and 40% reagent costing. Turn-around-time is reduced from 48 to 24 hours and timely reports facilitate appropriate therapeutic intervention and clinical management.

PS:3

Multiplex Real-Time PCR Detection of Three Drug-Resistant Mutations in Mycobacterium tuberculosis in a Single Tube with Displacing Probes

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The emergence of multidrug-resistant tuberculosis (MDR-TB) menaces the global TB-control efforts. Rapid detection of MDR-TB is urgent to prevent the spread of MDR-TB. We describe here a real-time method based on displacing probes for rapid screening streptomycin-, isoniazid- and ethambutol-related resistant Mycobacterium tuberculosis simultaneously in sputum samples. Three displacing probes labeled with FAM, HEX, and ROX, respectively, were used to detect the mutations in rpsL43 (AGC→ACC) and rrl315 (AGC→ACC) and embB306 (ATG→GTG), which are mostly associated with streptomycin, isoniazid, and ethambutol resistance, respectively. The method was capable to detect 10 copies of TB per reaction, and could identify all the three mutations simultaneously in the single reaction tube. The method was validated with 118 M. tuberculosis-positive sputum samples, among which we detected 8 samples to be streptomycin-resistant, 6 samples isoniazid-resistant, 1 sample ethambutol-resistant, 2 samples streptomycin- and isoniazid-resistant, and 8 samples streptomycin-, isoniazid- and ethambutol-resistant. The results of the real-time PCR assay were consistent with the conventional ARMS assay. Due to its rapidness (< 3 h), and high sensitivity, it could be valuable for high-throughout screening for the MDR-TB in clinical settings where real-time PCR machines were equipped.

PS:4

Pyrosequencing™ analysis of the gyrB gene to differentiate bacteria responsible for diarrheal diseases

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Abstract Pathogens causing acute diarrhea include a large variety of species from Enterobacteriaceae and Vibrionaceae. In China, the most common diarrheal bacteria, in order of prevalence, are Shigella spp., E. coli, Vibrio parahaemolyticus, Salmonella enterica, Vibrio cholerae, Aeromonas spp., Yersinia enterocolitica and Plesiomonas shigelloides. Rapid identification of causative bacteria with a high degree of specificity and sensitivity is essential to outbreak surveillance and investigation, and is also helpful in the implementation of proper clinical therapies. A method based on pyrosequencing™ was used here to differentiate bacteria commonly associated with diarrhea in China; the method is targeted to a partial amplicon of the gyrB gene, which encodes the B subunit of DNA gyrase. Twenty eight specific polymorphic positions were identified from sequence alignment of a large sequence dataset and targeted using 17 sequencing primers. Of 95 isolates tested, belonging to 13 species within seven genera,
most could be identified to the other \textit{E. coli} types; the genus \textit{Shigella}, except for \textit{S. boydii} and \textit{S. dysenteriae}, could also be identified; \textit{Salmonella} Enteritidis and Paratyphi A could be identified at the serotype level, while Choleraesuis, Paratyphi B, Typhi and Typhimurium could not be easily differentiated from other, uncommon serotypes, which are rarely isolated from fecal specimens in China, such as Agona, Mbandaka, and Kedougou etc. Considering the infrequency with which these serotypes are encountered in China, these limitations do not seriously compromise the validity of the current assay. All these isolates were also subjected to conventional sequencing of a relatively long (~1.2 kb) region of \textit{gyrB} DNA; these results confirmed those with pyrosequencing\textsuperscript{TM}. Twenty two fecal samples were surveyed, the results of which were concordant with culture-based bacterial identification, and pathogen detection limit with simulated stool specimens was 10\(^4\) CFU per ml for \textit{S. sonnei}. DNA from different pathogens \textit{S. Typhimurium} and \textit{S. Paratyphi A} was also mixed to simulate a case of multibacterial infection, and the generated signals correlated well with the mix ratio. In summary, the \textit{gyrB}-based pyrosequencing\textsuperscript{TM} approach proved to have significant reliability and discriminatory power for enteropathogenic bacterial identification. It is reasonably rapid: the results described here are available within 8 hrs, from DNA extraction of isolates or fecal samples to pyrosequencing and postsequencing analysis. It is cost-effective: cost analysis of the current assay comes to about $25 for each fecal sample. We believe the system is capable of rapidly and accurately tracking bacterial agents in outbreaks of diarrheal disease. \textbf{Key words:} \textit{gyrB}; Pyrosequencing; Enterobacteriaceae, \textit{Vibrionaceae}.

\textbf{PS:5} \hfill

\textbf{Multi-drug Resistance Profiles Correlate with Genetic Profiles in Clinical Isolates of \textit{Acinetobacter baumannii}} \hfill

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\textbf{Background:} \textit{Acinetobacter baumannii} is an important pathogen that is increasingly responsible for outbreaks of nosocomial infections worldwide including pneumonia, urinary tract infection, endocarditis, surgical site infection, meningitis, and sepsisemia. Previously susceptible to most antibiotics, \textit{A. baumannii} now exhibits resistance to a wide range of antibacterial agents including β-lactams, aminoglycosides, fluoroquinolones, chloramphenicol, tetracycline, and rifampin, the major antibiotic classes commonly used to treat these infections. We hypothesized that multi-drug resistance profiles in clinical isolates of \textit{A. baumannii} correlate with their genetic profiles. In this study, we examined antibiotic resistance and genetic profiles of \textit{A. baumannii} from Los Angeles County in an attempt to determine the correlation between antibiotic resistance profiles and genetic characteristics of the isolates. \textbf{Methods:} Minimum inhibitory concentration (MIC) assays were conducted based on microdilution procedures from Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) for sixteen breakpoint antibiotics against twenty clinical isolates of \textit{A. baumannii}. Antibiotic resistance profiles were established based on published breakpoint values. Plasmids were isolated using QIAGEN miniprep kits, digested with \textit{Hin}dIII and separated on 1% agarose gels. Genomic DNAs were isolated and were digested with restriction endonuclease A\textit{pal I}. Digested genomic DNA fragments were separated using a BioRad CHEF-DR\textsuperscript{III} system. \textbf{Results:} All 20 isolates were multi-drug resistant. Antibiotic resistance profiles correlate well with genetic profiles of plasmid and genomic DNA analysis. For example, 5 groups of isolates were evident based on plasmid and genomic DNA analysis: LAC 01-03; LAC 05, 08; LAC 07, 09, 10; LAC 11, 12, 13, 14; LAC 16-20. Isolates within each of these groups had similar antibiotic resistance (susceptibility) profiles. The remaining three isolates (LAC 04, 06 and 15) are each unique genetically and they all had unique susceptibility profiles. \textbf{Conclusions:} Using antibiotic susceptibility testing and molecular typing, we have confirmed our hypothesis that antibiotic resistance profiles correlate with genetic characteristics in these clinical isolates of \textit{A. baumannii}. This relationship may aid clonal analysis of clinical bacterial pathogens based on antibiotic susceptibility fingerprinting profiling. \textbf{Acknowledgments:} Funding for this project has been partially provided by NIH RIMI (R20MD001824) and NIH MBRS-SORE grants (S06GM008101) to H. H. Xu. We thank the MORE program of CSULA for additional support and the LA County Public Health Laboratory for supplying isolates.

\textbf{PS:6} \hfill

\textbf{Rapid genotyping of CTX-M extended-spectrum beta-lactamases by denaturing high-performance liquid chromatography} \hfill

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\textbf{Objective:} To develop a sensitive, rapid and high-throughput method using dHPLC (Wave\textsuperscript{®} Microbial Analysis System, Transgenomic) for genotyping CTX-M producing ESBLs. \textbf{Methods:} Thirteen well-defined \textit{bla\textsubscript{CTX-M}}-producing ESBL strains were used to develop and optimize the dHPLC genotyping assay. Four sets of multiplex primers were used to amplify fragments (255-293bp) of the ORFs of group 1, 2, 9 and 25/26/8 of \textit{bla\textsubscript{CTX-M}}. One strain from each group was selected as the reference and formed a homoduplex and a heteroduplex with the PCR amplicons representing the different members of \textit{bla\textsubscript{CTX-M}} genes from the relevant group. 6μl of the duplex samples were analysed on the Wave dHPLC system. The results were shown in the form of chromatographic peaks. Further evaluation was carried out with a blinded panel of 62 clinical isolates. 73 \textit{bla\textsubscript{CTX-M}}-ESBL-producing strains from the 4-month survey study were typed by the newly developed dHPLC genotyping method. The dHPLC genotyping results were further confirmed by DNA sequencing of a limited number of isolates. \textbf{Results:} The sequences of the PCR amplicons of 13 control strains were aligned and analyzed. Among 15 sequence variations within the PCR fragments from four \textit{bla\textsubscript{CTX-M}} groups, eight led to a change in the amino acid sequence and that seven were silent mutations. All samples were initially analyzed at a wider range of temperatures up to ±3°C of the predicted temperatures in order to achieve the best resolution of heteroduplexes. The characteristic chromatogram profiles for distinguishing \textit{bla\textsubscript{CTX-M}} member strains of all four groups were obtained. Applying the newly developed dHPLC-based geno-
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In today’s rapidly-changing world, new antimicrobial agents and emergence of resistance make paper journals and textbooks obsolete. www.antimicrobe.org is an internet-based database that focuses on infectious diseases and clinical microbiology. Five databases form the core of this website: pathogenic microbes, antimicrobial agents, infectious disease syndromes, infection control practice and HIV. Over 500 international authorities in epidemiology, infectious diseases, and pharmacology maintain and keep the databases up-to-date. Embedded links allow information transfer at the point of use for history of medicine vignettes, images of infectious diseases, pdfs of review articles, and thousands of abstracts and references; such a database would be prohibitive and unwieldy for print media. Given the magnitude of these databases, a Smart Search allows rapid searches of a specific topic; artificial intelligence techniques are applied, rather than keyword searches. The Guided Medline Search within the website allows retrieval of pertinent articles which have been published after the latest update of the chapter. In a small controlled study, Medline searches performed within the website produced more relevant search results than an individual using PubMed. A knowledge base of anatomy, microbiology, and immunology concepts support the basis for vignettes on pathogenesis that can be linked to the infectious disease under evaluation by the clinician. Likewise, a knowledge base of pharmacology supports the basis for translational concepts of optimal dosing and duration of antimicrobial therapy. Rapid delivery of information for epidemics, emerging pathogens, availability of new diagnostic methods and adverse effect warnings for antimicrobial agents can be made immediately available to the physician and clinical microbiologist. Moreover, customized information on antibiotic use that conforms to the antibiotic policy of an individual hospital can be targeted for physicians at the hospital where they practice. A Chinese version in both simplified script and traditional Chinese script is available for management strategies they practice. A Chinese version in both simplified script and traditional Chinese script is available for management strategies they practice. A Chinese version in both simplified script and traditional Chinese script is available for management strategies they practice. A Chinese version in both simplified script and traditional Chinese script is available for management strategies they practice. A Chinese version in both simplified script and traditional Chinese script is available for management strategies they practice.

Investigation of Increased Healthcare-Associated Urinary Tract Infections (UTI) Identified by Automated Surveillance

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Introduction: Indwelling urinary catheters are required for certain medical conditions. However, if overused, they can result in excess UTI. We saw an increase in UTI at our healthcare network in the spring of 2003 that was detected by automatic surveillance resulting in an Infection Control investigation. Methods: The MedMined™ Data Mining Surveillance Service® is a novel system that uses artificial intelligence, statistics and database technology to analyze large amounts of microbiology and hospital census data to generate organism or disease trends without manual entry or predefined search parameters. These trends (alerts) are reported monthly. We began populating our MedMined™ database 9/1/02. First alerts were reported 1/10/03. An increase in the number of alerts related to UTI was seen in April 2003. Alerts were investigated by chart review to determine if the UTI was catheter-related. Alerts were shared with the respective hospital unit and infection control practices reiterated. We discovered that a nursing policy for indwelling urinary catheter care did not exist. In response, we performed a point prevalence survey to determine appropriateness of catheter use. A physician/nurse and clerical assistant visited each patient. If catheterized, care was assessed by direct observation and indications for initial catheterization and continuation were determined by medical record review and consulting the patient’s nurse. Results: 658 patients were observed with 128 (19%) catheterized, including 48(38%) catheterized for inappropriate reasons. Overall, 62% were catheterized for >48hours and 31% had at least one issue with poor catheter care. A policy describing appropriate use and care was written but had little impact through January 2007. Subsequently, a new infection control program was proposed that would employ a nurse to review daily for appropriate use and directly prompt physicians regarding timely removal of a urinary catheter. Conclusion: The Data Mining Surveillance Service® is a potent tool for the detection and tracking of trends that permits focused investigation and intervention. This program lead to the institution of a novel infection control program that should reduce the number of hospital-acquired UTI.

Rigorous MRSA Surveillance Using RT-PCR: 22-Month Experience

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Background: Increasing incidence of methicillin-resistant Staphylococcus aureus (MRSA) is seen in many community & healthcare settings. Rapid detection is now possible using DNA-based real-time PCR that allows detection of the organism’s mecA gene in ~2 hours. Method: Active surveillance for MRSA using RT-PCR (BD-GeneOhm) began in the Neonatal Intensive Care Unit (NICU) at Newark Beth Israel Med Ctr, NJ, in March 2006, followed by the adult ICU and cardiac
units. According to the protocol, newly admitted patients (pts) to these units are put on isolation and tested for MRSA nasal colonization. Pts tested positive for MRSA DNA in their nares are treated with topical antibiotic (Mupirocin). All positive DNA results are followed by culture to determine antibiotic susceptibility. Follow-up data for 22 months are analyzed. Result: A total of 4,092 MRSA PCR tests from 3,743 pts were performed in 22 months of surveillance, of which 492 (12%) were positive. In addition, there were 19 (0.5%) unresolved PCR results even after repeat testing, while the remaining cases were negative. A decline in MRSA infection was evident upon the launch of the surveillance program. In our NICU, there were 2 infections in 22 months of active surveillance versus 20 infections in the 12 months preceding surveillance. In our adult ICU, a similar benefit was observed whereby MRSA infection declined to zero among transferred patients during the 6 months of surveillance. Of the 492 positive DNA tests, 296 (60%) were confirmed to be positive by culture [PCR+ culture+] while the remaining 196 (40%) failed to grow MRSA on culture [PCR+ culture-]. Most of the 196 [PCR+ culture-] cases represented follow-up testing of patients treated for previously positive nasal MRSA screen. Furthermore, 21 of the 196 [PCR+ culture-] nasal samples were found to have corresponding culture-positive MRSA originating from other body sites, e.g., wound, catheter tip & blood cultures. Some of these 21 pts were under antibiotic treatment at the time of PCR+ nasal screen, and probably represented true positive cases. Discussion: DNA-based molecular detection of MRSA enables same-day result compared to an average of 48 hours for traditional cultures. Rigorous MRSA surveillance has led to a significant reduction in MRSA infection in our institution. Used as a screening tool, MRSA RT-PCR detects more positive cases than culture, and appears to have superior sensitivity for samples with low bacterial load or a history of antibiotic treatment. Unlike cultures, DNA-based method has the advantage of detecting organisms inhibited by antibiotics. It remains undetermined how many cases, if any, were false positive. However, as a screening test, a certain degree of false positivity would be tolerable and relatively harmless from a clinical standpoint. Longer follow up is needed for more insight of the cost-benefit ratio of DNA-based MRSA surveillance on hospital admission.

PS:11

Epidemiological Profile of Nosocomial Infections in a Public sector Tertiary Care Hospital of Pakistan

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Studying prevalence of nosocomial infections is an effective predictor of managerial and hygienic quality as well as space constraints of a hospital. We conducted an observational study on a total of 32,620 patients who visited the Mayo Hospital, Lahore, Pakistan, from 1999 to 2004. These patients were assigned to different wards of hospital according to the nature of their disease, and were considered at maximum risk of contracting nosocomial infections due to patients saturation in the hospital. The clinical observations revealed that of the 32,620 patients, a total of 4502 (13.8%) contracted a disease other than their actual ailment. To gain information on prevalence and nature of pathogens involved in various types of nosocomial infections in these 4502 patients, clinical samples from these patients were collected avoiding contamination of different aetiologic agents. These clinical samples consisted of 1040 samples of pus & wound swabs, 109 of blood, 115 of pleural fluids, 286 of ascitic fluids, 37 of cerebrospinal fluid, 1398 of urine, 988 of sputum and endotracheal secretions, 329 of burn swabs, 99 of patients’ medical devices and 101 of fecal and drainage material. The presence of pathogens causing nosocomial infections was tested in these samples using isolation, biochemical and serological identification techniques. From a total of 4502 samples, Staphylococci were identified in 1287 (28.6%), Streptococci in 429 (9.5%), Enterococci in 328 (7.3%), Pseudomonas in 781 (17.3%), Enterobacter in 349 (7.8%), Acinetobacter in 41 (0.9%), Klebsiella in 266 (5.9%), Proteus in 140 (3.1%), Escherichia in 1031 (22.9%), Serratia in 67 (1.5%), Haemophilus in 93 (2%), other types of Gram positive bacteria in 119 (2.6%), other types of Gram negative bacteria in 13 (0.3%) and yeast and fungi in 189 (4.2%) samples. The overlapping of %age values indicate the presence of more than 1 or 2 pathogens in some samples, pointing out the patients’ saturation and poor hygienic quality in the hospital. These findings suggest for strict implementation of infection control measures to minimize patients’ saturation and to improve hygienic quality within the Mayo Hospital, Lahore, Pakistan.

Key Words: Epidemiological Profile, Nosocomial infections, Mayo Hospital Lahore Pakistan

PS:12

Importance of a Positive Stool-PCR Test in Detection of Helicobacter pylori Infection in Children

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Background/aim: Helicobacter pylori is usually acquired in childhood and is one of the most common bacterial infections in human. The purpose of this study was to evaluate clinical importance of stool PCR in H.pylori infected children.

Materials & methods: Based on endoscopic features and (or) a positive rapid urease test (RUT) in endoscopy room, a group of 35 children were selected to compare biopsy-based tests with stool PCR. Their first stool after endoscopy was stored at-70ºC, and their gastric activity and bacterial density were graded by updated Sydney system. Biopsies were cultured on modified campy-blood agar plates and colonies consistent with those of H. pylori were identified by biochemical tests and PCR using primers for detection of vacA, cagA, ureC and

Key Words: Epidemiological Profile, Nosocomial infections, Mayo Hospital Lahore Pakistan
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16srRNA genes. Substances in stool that inhibit PCR reaction were removed by filtration through a polypropylene membrane and an optimized protocol was used to detect H. pylori in stool using the same primers that were used for detection of H. pylori genes in biopsies. Results: Sixty-percent of the children were classified as H. pylori-positive by either a positive-culture or both positive RUT and histology. DNA from culture-positives showed amplification for vacA, cagA, ureC and 16srRNA. Stool-PCR was positive for 46% of the positive-controls; accordance was observed between the detection of H. pylori genes in biopsy and stool samples. Seventy-eight percent of stool-positive children were also positive by histology and association was observed between the positive-stool-PCR and higher score of H. pylori in histology. Conclusion: Specific detection of H. pylori DNA in stool is not only a useful test to detect H. pylori infection in children; it can give the information on the genotypes of strains present in the stomach. Association between higher score of H. pylori in histology and a positive stool-PCR make it a very useful test for detection of active infection and give the information on the degree of stomach colonization by H. pylori.

PS:13

Simultaneous Detection of Antibodies to Hepatitis C Virus Proteins Using the Luminex System to Distinguish Acute from Chronic HCV Infection

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Background: Infection by hepatitis C virus (HCV) is a leading cause of both end-stage liver disease and liver cancer. Identification of early infection is important because therapy initiated during the acute stage can reduce significantly the risk of developing chronic infection. To date, however, there are no reliable serological or virological assays to diagnose or screen for acute hepatitis C. Purpose: Our aim was to develop a high-throughput assay to measure differences in the anti-HCV IgG response elicited during the acute and chronic phases of infection. Methods: One hundred serum samples from 23 anti-HCV seroconversion panels (the “acute group”) obtained 1-65 days after the day that anti-HCV was last undetected, and 141 anti-HCV positive plasma specimens from blood donors (the “chronic group”) were assembled. All samples from the chronic group were confirmed for anti-HCV by the Ortho recombinant immunoblot assay and HCV RNA by reverse-transcriptase PCR. Three recombinant antigens, each originating from the core, NS4 and NS5 proteins, and 5 antigens derived from the NS3 protein (designated NS3#201, NS3#207, NS3#208, NS3#210 and NS3#215) were coupled to microspheres. IgG binding to the coupled antigens were assayed using the Luminex system. Two classifiers using logistic regression and decision trees (MATLAB) were built to confirm the accuracy of categorizing study samples as belonging to the acute or the chronic group. Results: In the acute group, the mean signal/cutoff (S/C) values were: core, 3.58 (95% CI: 2.93-4.23); NS3#201, 2.04 (1.32-2.75); NS3#207, 1.21 (0.96-1.46); NS3#208, 1.21 (0.84-1.57); NS3#210, 1.67 (1.14-2.21); NS3#215, 1.67 (1.20-2.15); NS4, 2.07 (1.60-2.54); and NS5, 1.29 (1.00-1.58). In the chronic group, the mean S/C values were: core, 13.10 (95% CI: 12.50-13.69); NS3#201, 10.23 (8.91-11.55); NS3#207, 4.61 (3.93-5.29); NS3#208, 3.93 (3.15-4.70); NS3#210, 17.50 (14.96-20.04); NS3#215, 13.19 (11.02-15.36); NS4, 12.00 (10.82-13.17); and NS5, 8.27 (6.75-9.79). Differences in mean S/C values between the acute and chronic groups were all significant (P<0.001). The stratified 10-fold cross-validation accuracies of the two classifiers were 95.8% +/- 2.07% and 90.3% +/- 3.11% for logistic regression and decision tree analysis, respectively. Conclusions: These data suggest that the multiplexed HCV recombinant-antigen microsphere immuno- assay is a viable tool to identify acute hepatitis C.

S6:1

Developing a Plan for Introducing a New Program

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Introducing a major new practice to a healthcare system is a major organizational challenge. The example for this discussion is our experience with methicillin-resistant Staphylococcus aureus (MRSA). MRSA is now endemic in many hospitals in the United States hospitals. Colonization with this organism is associated with increased risk of disease, which is associated with high cost and poor clinical outcomes. In 2002 we began to develop a plan for surveillance of patients to detect MRSA colonization. The program began with a risk assessment consisting of a point prevalence survey in August 2004 that found 8.5% of inpatients colonized with MRSA; 2/3 of which were not previously known to the infection control program. Once the risk was deemed to be substantial, a leadership team was created in January 2005. This consisted of a health care epidemiologist, the senior vice-president of nursing, the senior vice president of quality, and one of the three hospital presidents. Members were also drawn from infection control, microbiology, nursing, medicine (program design and validation), nursing (program implementation), information systems (software design), information technology (systems design), building management (program capacity), environmental services (isolation capacity), pathology and laboratory medicine (workflow), staffing and testing, and finance (cost and benefit analysis). Frequent multidisciplinary meetings were held to design, plan, and launch the initiative. The health care epidemiologist developed the program, including informational handouts for healthcare staff and patients about MRSA; an instructional document and training video on how to swab the nose, and the design of order sets for MRSA surveillance swabbing as well as for the de-colonization protocol. A series of strategies was developed to ensure maximal buy-in before rolling out universal surveillance including recruitment, education, and involvement of nursing leadership at each hospital in the program; promulgation of educational documents and video for staff and patients; grand rounds for physicians; documented education with competency evaluations for the patient care technicians who were collecting the swabs; streamlined computerized test order entry; development of a test kit that included a swab and instructions placed in each patient room prior to admission; and real-time surveillance compliance with feedback and education to nursing units with suboptimal testing rates. Before roll out of the program, goals were developed by the initial leadership team to be used in measuring success that were included in the business plan.
The two primary goals were to demonstrate a clinical benefit by reducing MRSA infection and to be at least cost neutral.

S6:3

Emerging Technology - Critical factors for application in routine point of care testing

**G. H. Thorpe: Gary Thorpe Associates Limited, Birmingham, UNITED KINGDOM**

A wide range of point of care (POC) testing systems is commercially available world-wide. These cover applications in diverse areas and are based on numerous established or emerging technologies. The systems differ widely in complexity and range from disposable, single use non-instrumental systems to integrated systems designed for rapid detection of biohazards by automated DNA detection. POC systems are used in a range of extra-laboratory situations, include qualitative, semi-quantitative and fully quantitative devices, and can produce a single result or a panel of multiple, simultaneous results. Results can be indicated by various means including the production of coloured responses of varying intensity, symbols or words, or by conventional digital readout. Several factors are critical for the application of technologies in routine POC testing. Irrespective of the complexity of technologies, systems should be; simple to use, based on ‘self contained’ stable reagents, conveniently stored, and designed to minimise operator dependent steps such as timings, volume pipetting, sequential procedural steps, calibration, and subjective determination of results and their interpretation. POC systems incorporating emerging or established technologies should also enable production of appropriate cost and ‘clinically effective’ results for specific applications, with the system’s characteristics including identification of suitable samples, performance and limitations clearly identified in the product’s claims and instructions for use. The POC market is currently dominated by systems for use in blood glucose monitoring and by single use immunoassay devices. However, an enormous number of POC microbiological tests are currently becoming available. The introduction of ‘miniaturised and packaged’ molecular biology based systems, encouraged by a need for systems for bio-warfare/bio-terrorism detection of chemical and biological agents, offer exciting opportunities for rapid and sensitive assays and detection of infectious agents. The introduction of new technologies and assays offers extremely exciting prospects and challenges for POC diagnostics.

S7:1

Molecular Diagnosis of Fungal Pathogens

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Infections caused by fungi have increased in the recent decades. Accurate and rapid identification of fungal pathogens is important for appropriate treatment with antifungal agents. Based on the internal transcribed spacer 1 (ITS1) and 2 (ITS2) sequences of the rRNA genes, three oligonucleotide arrays were developed to identify 64 species (32 genera) of filamentous (or dimorphic) fungi, 77 species (16 genera) of yeasts, and 17 species of dermatophytes, respectively. The methods consisted of PCR amplification of the ITS regions using a pair of universal primers, followed by hybridization of the digoxigenin-labeled PCR products to a panel of species-specific oligonucleotide probes immobilized on a nylon membrane. Sensitivity and specificity of >97% were obtained by the three arrays for identification of pure cultures. Furthermore, our studies revealed that identification of medically important yeasts and dermatophytes by ITS sequencing is also very reliable and can be used as an accurate alternative to conventional identification methods. In conclusion, the present methods (array hybridization and ITS sequencing) are powerful tools for identification of clinically important filamentous fungi and yeasts. The whole procedures can be finished within 24 h starting from isolated colonies.

S7:4

Rapid and On-demand Detection and Characterization of Staphylococci Causing Bloodstream Infections


Phenotypic methods take several days for identification and antimicrobial susceptibility testing of staphylococcal isolates after gram-positive cocci in clusters (GPCC) are observed in positive blood cultures. We developed and validated a StaphPlex system for species-level identification of staphylococci, detection of genes encoding Panton-Valentine leukocidin (PVL), and antimicrobial resistance determinants of staphylococci. The StaphPlex system was compared to phenotypic methods for organism identification and antimicrobial resistance detection for positive blood culture specimens in which GPCC were observed. Among a total of 360 GPCC specimens, 273 (75.8%), 37 (10.3%), 37 (10.3%), 1 (0.3%), 3 (0.8%), and 9 (2.5%) were identified by StaphPlex as coagulase-negative *Staphylococcus* (CoNS), methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *S. aureus* (MSSA), or mixed infections of CoNS and MRSA, CoNS and MSSA, or non-staphylococci, respectively, with an overall accuracy of 91.7%. The 277 CoNS-containing specimens were further identified to the species level with an overall accuracy of 80.1% compared to a combined reference identification. High very major errors were noticed when detection of *aacA, ermA, ermC, tetM*, and *tetK* was used to predict in vitro antimicrobial resistance, but relatively few major errors were observed when the absence of these genes was used to predict susceptibility. The StaphPlex system demonstrated high sensitivity and specificity when used for staphylococcal cassette chromosome mec typing and PVL detection. StaphPlex provides simultaneous staphylococcal identification and detection of PVL and antimicrobial resistance determinants within 5 h, significantly shortening the time needed for phenotypic identification and antimicrobial susceptibility testing.
Validation of a Virulence and Epidemiology (VirEp) DNA microarray for *Staphylococcus aureus*

R. P. Spence, V. Wright, D. A. Ala’Aldeen, K. G. Wooldridge, D. P. Turner; R. James;
University of Nottingham, Nottingham, UNITED KINGDOM.

The human pathogen *Staphylococcus aureus* is isolated and characterized using traditional culture and sensitivity methodologies that are slow and offer limited information on the organism. In contrast, DNA microarray technology can provide detailed clinically-relevant information on the isolate by detecting the presence or absence of a large number of virulence associated genes simultaneously in a single assay. We have developed an oligonucleotide microarray assay for identification and characterization of *Staphylococcus aureus*, including key virulence determinants. The multi-well format microarray is capable of examining 13 different isolates simultaneously, together with a reference control strain. The microarray comprises 84 gene targets including species-specific, antibiotic resistance, toxin and other virulence-associated genes.

Analysis of *S. aureus* isolates whose complete genome sequence has been determined (Mu50, N315, MW2, MRSA252, MSSA476) demonstrated that the array can reliably detect the combination of genes known to be present in these isolates. Characterization of a further 43 *S. aureus* isolates by microarray and PFGE has demonstrated the ability of the array to differentiate between isolates representative of a spectrum of *S. aureus* types including MSSA, MRSA, CA-MRSA and VRSA and simultaneously detect clinically relevant virulence determinants.

Analysis of 18 outbreak *S. aureus* isolates, based on the presence or absence of the 84 target genes, indicated that the isolates clearly grouped into two genotypes that were consistent with those determined by PFGE. The VirEp microarray thus represents a new tool for the characterization of clinical *S. aureus* isolates that also allows their rapid epidemiological investigation.

**POSTER ABSTRACTS**

**1A**

The Transcriptome of *Chlamydia trachomatis*
Persistent Infection: Identification of Novel Targets for Nucleic Acid Amplification Tests

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*Chlamydia trachomatis* is an obligate intracellular pathogen and a major cause of sexually transmitted infection (STI). It is thought that persistent infection plays an important role in sustaining chronic inflammatory responses that lead to damaging sequelae such as pelvic inflammatory disease. Persistent infection is modeled in *vitro* by treatment of infected epithelial cells with interferon-gamma (IFN-γ) that transforms normal chlamydial developmental growth forms into large morphologically aberrant non-infectious organisms. These aberrant persistent forms reanimate into developmentally normal infectious organisms following removal of IFN-γ. This pathogen specific sensing and modulation of its normal developmental biology in response to IFN-γ likely evolved as a strategy to avoid the host immune response. In this study we have characterized the chlamydial transcriptome of IFN-γ persistent growth to better understand the genetic and biological basis of persistence and identify novel targets with potential utility in the design of new nucleic acid amplification tests (NAATs) for diagnosing chlamydial infection. We found marked coordinated differences in the transcriptional profiles of normal and persistent growth forms. Of particular interest was the identification of a subset of highly up-regulated genes specific to persistently infected cells. These included genes involved in tryptophan biosynthesis, DNA recombination and repair, phospholipid utilization, protein translation, and general stress responses. We suggest that these here-to-for unrecognized highly up-regulated transcripts such as *trpB* might be superior targets for NAATs that offer enhanced sensitivity for diagnosing chlamydial infection.

**2B**

Detection of TEM-derived ESBLs in *E. coli* and *K. pneumoniae* of 19 hospital in China

W. Yao, X. Yingchun, Y. Qiwen, X. Xiuli, C. Minjun; Peking Union Medical College Hospital, Beijing, CHINA

Abstract: **Objective** To detect TEM-derived ESBLs in *E. coli* and *K. pneumoniae* of 19 hospitals in China. **Method** 98 *E. coli* and 109 *K. pneumoniae* resistant to ceftazidime were collected from 2004 to 2005 in 19 hospitals in China. Their MICs were detected by agar diffusion method. Further analysis was done by PCR, conjugating experiment, isoelectric focusing, DNA sequencing and DHPLC. Then collected all the strains isolated from the host whose strains expressing TEM-derived ESBLs and the strains non-susceptible to ceftazidime isolated from the same ward, analyzed them by DNA sequencing and DHPLC, and typed by ERIC-PCR and RAPD. **Results** There were 84.7% *E. coli* and 68.8% *K. pneumoniae* producing ESBLs, and 67.3% *E. coli* and 69.7% *K. pneumoniae* expressed *blaTEM*. Only 1 *E. coli* P34 expressed TEM-12, all the others expressed TEM-1. In the 21 *E. coli* isolated from the host of P34, there were 18 strains expressed TEM-12, and all the strains belonged to the same clone. All the 11 *E. coli* non-susceptible to ceftazidime in the same ward expressed TEM-1, and belonged to different clones with P34. But there were still clone-spreading between different patients. **Conclusions** TEM-derived ESBLs were still rare in China, and they weren’t the main reason for the resistance to ceftazidime. This is the first report of TEM-12 in China. **Key words:** *E. coli, K. pneumoniae,* TEM-derived ESBLs

**3C**

**POSTER ABSTRACTS**

**4B**

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**3C**

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Analysis of 18 outbreak *S. aureus* isolates, based on the presence or absence of the 84 target genes, indicated that the isolates clearly grouped into two genotypes that were consistent with those determined by PFGE. The VirEp microarray thus represents a new tool for the characterization of clinical *S. aureus* isolates that also allows their rapid epidemiological investigation.

**4D**

The Diagnostic Potential of miniaturised DNA microarrays.

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We have developed two sensitive and specific miniaturized microarray chips suitable for high throughput use for detection of virulence and antimicrobial resistance (AMR) genes.
The virulence gene chip contains 63 oligonucleotide probes representing 6 different Escherichia coli pathotypes, whilst the AMR gene chip contains 60 probes representing 45 different resistance groups present in Gram-negative bacteria against antibiotics commonly used for treating infections caused by enteric bacteria. The probes used on each chip and the assay was validated using sequenced control strains or genes. The virulence chips were used to pathotype E. coli clinical isolates of human and animal source. The data showed that strains of EHEC and EPEC pathotypes harbour similar virulence characteristics with presence of the stx toxin genes being the main differential. Whereas isolates characterised as UPEC, ETEC, EHEC or EAEC tended to show distinct virulence profiles. For the AMR chip presence of resistance genes in a panel of E. coli and Salmonella clinical isolates of human and animal origin was tested. The mean number of resistant genes present in E. coli isolates was found to be 8 and in Salmonella, 5. The most common gene detected in both was the extended β-lactamase gene blatem, which was present in 90 and 56% of the isolates tested, respectively. There was a good correlation between geno- and phenotypic results tested by broth microdilution using Sensititre plates or disc diffusion. The results demonstrated that these arrays provide an effective, economic and simple method for high through-put detection of known virulence and resistances circulating in Gram-negative bacterial populations that are of clinical relevance. Newly emergent virulence and AMR genes will be detected by addition of probes in an iterative manner to the chips in future.

5A
The potential for little egret to serve as a reservoir and vehicle for Laribacter hongkongensis


Aims The isolation of Laribacter hongkongensis from freshwater fishes between Hong Kong and Hangzhou City led us to investigate the possible reservoir and vehicle of this bacterium in the environment. It was first reported that Laribacter hongkongensis strains were isolated in feces of little egret (Egretta garzetta) from three habitats in Hangzhou City. Materials and Methods Fecal specimens of aquatic birds (>90% Little Egret; some Cattle Egret and Chinese Pond-Heron) were collected from three sites in Hangzhou City. A total of 169 individual specimens were isolated and identified as L. hongkongensis. All 169 fecal specimens were collected during June to July 2007. The bacteria were isolated and identified by phenotype tests, specific primers PCR, and 16S rRNA gene sequencing. The antibiotic resistance of isolated strains was also tested. Results L. hongkongensis was isolated from 10 (5.92%) of 169 fecal specimens, including 5 strains (7.25%) from Habitat A, 1 (2.0%) from Habitat B and 4 (8.0%) from Habitat C. Ten trains of L. hongkongensis tested are resistant to 13 of 24 antibiotic drugs. The rates of antibiotic resistance were 100% for Penicillin, Cefazolin and Cefoperazone. Discussion The results presented here indicate that little egret may serve as a reservoir and vehicle of L. hongkongensis. The organism was isolated from 10 (5.92%) of 169 freshly voided droppings specimens collected from three little egret habitats in Hangzhou City. But the source of infection of the birds with L. hongkongensis strains remains unknown. The birds may have become infected with the organism while feeding in some bodies of water that was contaminated with fecal material from animals or humans carrying L. hongkongensis. Migratory birds may have transported the organism to the other places from a focus where L. hongkongensis persists. It has been speculated that birds can contaminate the bodies of water with L. hongkongensis and that these bacterium are potentially a source of infection for fishes and humans. Conclusion The first isolation of L. hongkongensis strains from little egrets is reported. The results of this study suggest that little egret may serve as a reservoir and vehicle for L. hongkongensis.
Emerging Technologies of Medical Importance

**A simple and rapid lyophilized reagent for the simultaneous detection of Salmonella enterica, S. Typhi and S. Paratyphi**

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**Background:** Typhoidal diseases, mainly caused by *Salmonella enterica* serovars Typhi and Paratyphi A, are major enteric problems especially in developing countries. Molecular techniques especially PCR ensure an early and precise diagnosis of typhoid with superior sensitivity and specificity. However, since several serotypes are involved, a method for simultaneous detection of these pathogens is needed for differential diagnosis. Methods: To address this problem, a regular multiplex PCR targeting tyvelose epimerase (*tyv*) (previously called *rfbE*), *flcC*-a, paratose synthase (*prl*) (previously called *rfbK*), *vibB* and *flC*-a (phase-1 flagellin; Hα) genes has been previously developed. However, the scope of this multiplex PCR, though excellent in execution, is limited only to isolated bacterial cultures. It is not sensitive enough to directly detect very small number of bacteria that are circulating in blood of patients suffering from enteric fever. Its utility is not only limited by the time consumed involving blood culture and isolation of bacteria, but also by poor sensitivity of blood culture as it is positive in only 50% cases at most. We have extended this procedure and developed a nested multiplex PCR for differential diagnosis of typhoidal pathogens. The technique has been optimized to be directly applicable on clinical blood samples. Results: The results of nested multiplex PCR showed that out of 30 *Salmonella* isolates of 21 were Vi-positive *S. Typhi* (having *tyv*, *vibB*, *flC*-a and *prl* genes), 6 Vi-negative *S. Typhi* and 3 *S. Paratyphi* A (having *prl* and *flC*-a genes only). Out of 60 clinically suspected blood samples, 42 were found positive for *Salmonella*. Twenty six patients were found to be infected by Vi-positive *S. Typhi*, 9 with Vi-negatively *S. Typhi* and 2 with *S. Paratyphi* A. Five patients were found to have been mixed infection of Vi-positive *S. Typhi* and *S. Paratyphi* A. Several common pathogens were included as controls and all gave negative results. These included isolates of *Escherichia coli*, *Proteus*, *Mimececa*, *Staphylococcus*, *Streplococcus* and *Bacillus* spp.

Conclusions

We conclude that this technique can very rapidly and efficiently diagnose and discriminate among typhoidal pathogens directly from blood samples.

**Specific detection of drug resistance mutations in cytomegalovirus using real-time PCR**

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Ganciclovir (GCV) is a nucleoside analogue that suppresses the replication of cytomegalovirus by inhibiting viral DNA polymerase and it has been used as a primary antiviral agent for treating CMV infection. However, long-term GCV therapy induces the emergence of viral mutants that exhibit changes in the gene UL97 gene and the UL54 gene of CMV DNA. Since culture-based resistance screening is not a practical method for routine use, a method for detecting CMV mutations undertaken by real-time amplification refractory mutation system PCR (real-time ARMS PCR) using SYBR Green fluorescent dye was developed. The real-time ARMS PCR approach is described using specific primers to detect mutation in codon 460 of the UL97 gene with melting point analysis. The success of the method relies on the detection of a resistance-specific nucleotide by designed primers. These primers allowed specific amplification of the target sequence followed by SYBR Green binding and monitoring of PCR product accumulation. The feasibility of real-time ARMS assay of mutations in codon 460 was demonstrated using the artificial constructed plasmid of pUC19-M460V, pUC19-M460I and pUC19-wild. Discrimination between wild-type and mutant templates was demonstrated as the primer generate specific increasing fluorescence with their respective targets but only with those that were designed to anneal with the templates perfectly. Although amplification of the wild-type template with the mutant

**8D**

**Differential diagnosis of pathogens causing enteric fever directly from blood samples by nested multiplex PCR**

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**Background:** Typhoidal diseases, mainly caused by *Salmonella enterica* serovars Typhi and Paratyphi A, are major enteric problems especially in developing countries. Molecular techniques especially PCR ensure an early and precise diagnosis of typhoid with superior sensitivity and specificity. However, since several serotypes are involved, a method for simultaneous detection of these pathogens is needed for differential diagnosis. Methods: To address this problem, a regular multiplex PCR targeting tyvelose epimerase (*tyv*) (previously called *rfbE*), *flC*-a, paratose synthase (*prl*) (previously called *rfbK*), *vibB* and *flC*-a (phase-1 flagellin; Hα) genes has been previously developed. However, the scope of this multiplex PCR, though excellent in execution, is limited only to isolated bacterial cultures. It is not sensitive enough to directly detect very small number of bacteria that are circulating in blood of patients suffering from enteric fever. Its utility is not only limited by the time consumed involving blood culture and isolation of bacteria, but also by poor sensitivity of blood culture as it is positive in only 50% cases at most. We have extended this procedure and developed a nested multiplex PCR for differential diagnosis of typhoidal pathogens. The technique has been optimized to be directly applicable on clinical blood samples. Results: The results of nested multiplex PCR showed that out of 30 *Salmonella* isolates of 21 were Vi-positive *S. Typhi* (having *tyv*, *vibB*, *flC*-a and *prl* genes), 6 Vi-negative *S. Typhi* and 3 *S. Paratyphi* A (having *prl* and *flC*-a genes only). Out of 60 clinically suspected blood samples, 42 were found positive for *Salmonella*. Twenty six patients were found to be infected by Vi-positive *S. Typhi*, 9 with Vi-negativity *S. Typhi* and 2 with *S. Paratyphi* A. Five patients were found to have been mixed infection of Vi-positive *S. Typhi* and *S. Paratyphi* A. Several common pathogens were included as controls and all gave negative results. These included isolates of *Escherichia coli*, *Proteus*, *Mimececa*, *Staphylococcus*, *Streplococcus* and *Bacillus* spp.

Conclusions

We conclude that this technique can very rapidly and efficiently diagnose and discriminate among typhoidal pathogens directly from blood samples.

9A

Specific detection of drug resistance mutations in cytomegalovirus using real-time PCR
specific primer was not eliminated completely, the selectivity of the method could be determined easily using melting curve analysis. The sensitivity of the real-time ARMS PCR assay for M460V/I was 10^3 copies/ml. The specificity of the real-time PCR method described in this study for the detection of mixtures was examined by using defined mixtures of plasmids. In a mixture of defined proportion of mutant and wild-type templates, it was possible to detect 20% of the mutant or wild-type strain, respectively. In contrast to the conventional PCR-RFLP which is frequently time consuming and laborious, a real-time ARMS PCR method allows automated detection of point mutation without the need for post-amplification processing. This study demonstrated a rapid, highly sensitive and reproducible method for quantifying the mutant CMV in GCV treated patients. It can be used to monitor patients before and during GCV therapy. In comparison to other real-time PCR assays using molecular beacons or an hybridization probe, this approach can not only detect the mutation in condon 460, but also discriminate the precise mutation type.

10B

IsoAmp Molecular Analyzer, a disposable molecular diagnostic device for pathogen detection

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It is widely recognized that nucleic acid amplification based molecular diagnostic tests are more sensitive and specific than immunological tests. Unfortunately, molecular tests are more difficult to execute, and require dedicated instrumentation. We have developed an IsoAmp® Molecular Analyzer which can be used to perform nucleic acid test without any special instrument. The IsoAmp Molecular Analyzer consists: 1) upstream manual sample prep; 2) isothermal DNA amplification; and 3) rapid amplicon detection using the BES™ (BioHelix ExpressStrip) Cassette. Following isothermal amplification, the reaction tube is directly transferred to the BES™ cassette. The amplicon is detected on a DNA test strip without opening the tube, eliminating amplicon cross contamination. IsoAmp tests can achieve single-copy detection sensitivity, driven by isothermal Helicase-Dependent Amplification (HDA), which uses a helicase to separate duplex DNA eliminating complicated thermocycling. This simple and “instrument free” molecular diagnostic system offers an affordable and flexible solution for detecting pathogens at molecular level. Several IsoAmp assays are being developed for detecting a variety of microbial pathogens, including the IsoAmp Rapid Staph assay for the detection of Staphylococcus aureus and methicillin resistance Staphylococcus aureus (MRSA). Analytical sensitivity of the IsoAmp Rapid Staph assay was determined at 50 copies for both nuc and mecA genes. The IsoAmp Rapid Staph assay was validated on 119 Gram-positive cocci in clusters-containing blood culture medium specimens, which were collected from the Clinical Microbiology Laboratory at Vanderbilt University Medical Center and have been well characterized by biochemical and genotypic methods. The IsoAmp assay detected nuc gene in 39 of the 39 specimens previously tested positive for S. aureus, and in none of coagulase-negative staphylococci (CoNS) samples, giving 100% sensitivity and specificity. The assay detected mecA in all 19 blood specimens containing MRSA and in two specimens in which methicillin-sensitive S. aureus and methicillin-resistant CoNS were mixed, giving 100% sensitivity and 98% specificity.

11C

Investigation of nosocomial infections by AFLP

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Nosocomial (hospital acquired) infections are widespread. They are important contributors to morbidity and mortality, and increase the length of hospital stay and treatment costs. The majority of nosocomial infections are due to opportunist pathogens, which cause diseases only in severely debilitated patients. Knowledge about the epidemiology of these infections is important to improve control measures. The availability of new and rapid molecular fingerprinting techniques improves strain delineation, that is essential in establishing the epidemiologic characteristics of nosocomial infections. Epidemiologic typing systems can be used to investigate the clonal origin of pathogens involved in outbreaks. For surveillance systems, monitoring clonal spread and prevalence in populations over extended periods of time requires standardized and high throughput library typing systems. In this study, we report the results of a surveillance program involving 17 different high risk wards of Florence (Italy) hospitals. Over a period of 16 months, 2213 bacterial isolates, belonging to 15 different species, among the most common opportunistic pathogens, have been typed by Amplified Fragment Length Polymorphism (AFLP). This highly reproducible and discriminatory fingerprinting technique was used with the aim to determine the genetic relatedness of clinical and epidemiological relevant isolates. AFLP results were correlated with clinical and epidemiological data to analyse the permanence over the time and the spread of different clones within the hospital wards under investigation. The population structure of the predominant microorganisms, Staphylococcus aureus (22% isolates), Pseudomonas aeruginosa (25%), E. coli (13%) and Staphylococcus epidermidis (5%) has been determined, regarding the consistence and dynamics of resistant strains and clonal groups. Furthermore, the results confirmed the clonal origin of outbreaks of different species of pathogens and, conversely, verified suspect outbreaks by identifying the corresponding isolates as a clonal group. This study points out the importance of molecular typing systems to control the spread of bacteria in hospital setting and in the assessment of strategies to prevent development of nosocomial infections by defining the source of infection and the chain of pathogens transmission.
Emerging Technologies of Medical Importance

12D

MVPlex Assay for Simultaneous Detection and Differentiation of Methicillin-Resistant Staphylococcus aureus and Vancomycin-Resistant Enterococci from Nares and other Swab Specimens

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Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) are well-known causes of hospital-associated nosocomial infections and contribute to significant morbidity and mortality in infected hospitalized patients. Better, and faster, detection of MRSA and VRE allow the healthcare provider to more efficiently prevent the spread of nosocomial infections caused by the two pathogens. We evaluated an MVPlex assay (Geneco Biomedical Products, Inc., Huntsville, AL), which uses a target-enriched multiplex (TEM)-PCR for the simultaneous detection and identification of MRSA and VRE from 307 dual-swab specimens. The sources of specimens were evaluated from the ear (1.5%), nares (76.2%), rectum (2.3%), and the skin (20.2%). Nucleic acids from these specimens were extracted using a bioMérieux easyMAG, amplification of the nucleic acid target regions within nuc, mecA, SCCme-orfX (SRE), vanA, vanB, ddi, and inf genes was done using the TEM-PCR, and amplified products were detected and characterized using a microsphere suspension array on a Luminex 100 instrument. DNA extracts that tested positive for nuc, mecA, and SRE genes were considered MRSA. DNA extracts that tested positive for vanA or vanB gene were considered VRE. The MVPlex assay detected MRSA in 97 of the specimens while culture detected MRSA in 79 of the specimens. Out of 234 nares specimens, 26.5% were positive in culture and 33.3% were positive in the MVPlex assay. Out of 62 skin specimens, 25.8% were positive in culture and 29.0% were positive in the MVPlex assay. Out of 4 ear specimens, 25.0% were positive in culture and 25.0% were positive in the MVPlex assay. None of the 7 rectal specimens were positive for MRSA in either assay, VRE was detected in 1 (14.3%) of rectum specimens. Using a combination of culture and a BD GeneOhm™ MRSA assay as the “gold standard”, the MVPlex assay had a sensitivity of 97.8% and a specificity of 95.8% for MRSA detection. The entire MVPlex procedure, from specimen processing to result reporting, can be completed in five hours. These data indicate that the MVPlex assay provides a rapid and sensitive tool for MRSA and VRE dual screening directly from swab specimens.

13A


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Background: Worldwide resistance in enterobacteriaceae is increasing. Especially, resistance conferred by mobile genetic elements is of concern for patient safety. ESBL are the most important resistance mechanisms of this kind. Therefore reliable detection of ESBL in the laboratory is important for control strategies. Objective: To evaluate the positive predictive value of the Vitek2 (BioMerieux, Lyon, France) in combination with the advanced expert system for the detection of ESBL in E. coli and Klebsiella spp.

Method: During a two year period all E. coli and Klebsiella spp. isolated in routine clinical practice of a microbiology laboratory of a large teaching hospital were included. Per patient only one strain per species was included. Isolates that were suspect of ESBL production by the Vitek2 (card N048) were confirmed by Etest using cefotaxim (CT)/ceftotaxim+clavulanic acid (CTL) and cefazidine (TZ)/cefazidine+clavulanic acid (TZL). Susceptibility tests were performed according to the guidelines from the Clinical and Laboratory Standards Institute (CLSI). ESBL production was defined as recommended by the manufacturer (AB BIODISK): MIC ratio CT/CTL ≥ 0.5 and CT versus CT/CTL ≥ 8 and/or TZ/CT ≥ 1 and TZ versus TZ/TZL ≥ 8 and/or a “phantom” zone and/or deformation of the CT or TZ ellipse. Results: A total of 328 isolates were suspected of ESBL production: 272 E. coli and 56 Klebsiella spp. The Vitek2 result was confirmed by E-test in 158 (58%) of the E. coli strains and 24 (43%) of the Klebsiella spp. strains. The positive predictive value of the Vitek 2 was significantly lower in Klebsiella spp. than in E. coli (p=0.04). Conclusion: The reliability of ESBL detection by the Vitek2 in combination with the Advance Expert System was low in both species and requires confirmation. In Klebsiella spp. the positive predictive value was significantly lower than in E. coli.

14B

Multiplex Technology for Detection Respiratory tract Infection in Clinical Specimens

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Background: Respiratory tract infections are a significant cause of morbidity and mortality in young children, elderly subjects, and immunocompromised patients. Rapid diagnosis is important to patients on admission and implement proper infection control measures. Epidemic respiratory infections can be caused by a wide variety of pathogens, including bacteria, Mycoplasma pneumoniae, Chlamydia pneumoniae, or viruses such as influenza virus, adenovirus, rhinovirus, or coronaviruses et al. Although various culture methods, molecular techniques, and serologic diagnostic tests exist, for many epidemics the causative microorganism(s) are never determined. Furthermore, there has been no practical method for examining the broad pathogens ecology of respiratory infections to dissect the complex polymicrobial interactions that occur during explosive outbreaks of disease. Methods Using specific tiny microspheres, multiplex PCR technology and Luminex xMAP (flexible Multi-Analyte Profiling) have combined for rapid detection of 14 respiratory pathogens (18 typing) by DNA or RNA. Results The specificity of the diagnostic system have been validated by 15 pathogens (19 typing) from ATCC. There are not cross-reaction in each other. In 138 samples collected from clinical respiratory tract infections, Bacteria were detected in 26 (37.68%) of 69 pathogens from
112 bronchoalveolar lavage, including 13 (18.84%) *M. tuberculosis* infections and viral pathogens were detected in 44.93%, *M. pneumoniae* and *C. pneumoniae* were in 17.39%. Influenza A virus detected in bronchoalveolar lavage and 26 nasopharyngeal swab was 21 of 112 (18.75%) and 7 of 26 (26.92%) by flexible Multi-Analyte Profiling. Conclusion: Luminex xMAP Multi-Analyte Profiling were highly sensitive and accurate, high throughput and increased assay speed for detecting multiple respiratory pathogens in clinical specimens. It is useful tool for epidemiology yet.

15C

In vitro Activity of Tigecycline Against Gram-positive and Gram-negative Clinical Isolates from Tigecycline Evaluation and Surveillance Trial (TEST program 2007) in Hong Kong

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Background: Tigecycline belongs to a new class of antibiotics, glycyclcline. In this study (TEST program at Hong Kong center), antimicrobial activity of tigecycline against a variety of pathogens derived from clinical isolates collected at Prince of Wales Hospital during 2007 was evaluated. Methods: A total of 65 Gram-positive and 135 Gram-negative pathogens were collected and broth dilution MICs were determined using CLSI guidelines and FDA interpretive criteria. Results: Tigecycline demonstrated excellent activity against Enterobacteriaceae (n=85) with MIC₉₀ ≤1μg/ml. MIC₉₀ of *Escherichia coli* (n=25), *Klebsiella* spp. (n=25) and *Enterobacter* spp. (n=25) was ≤0.5μg/ml. In addition, it revealed potent activity against *Enterococcus* spp. (n=15), *Staphylococcus aureus* (n=25), *Streptococcus agalactiae* (n=10) and *Streptococcus pneumoniae* (n=15) with MIC₉₀ ≤0.25μg/ml. It was also the most active antimicrobial agent against multi-drug resistant *Acinetobacter* spp. (n=15). All clinical isolates tested were susceptible to tigecycline. Conclusion: Tigecycline showed potent antimicrobial activity against various Gram-positive, Gram-negative and even multi-drug resistant pathogens. It may be a new alternative agent for treatment of serious infections.

16D

Comparative study of phenotypic and genotypic methods for the detection of predominant ESBL and other emerging beta-lactamases producing isolates in a Veteran Medical Center in southern Taiwan

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Background: In recent years, extended-spectrum beta-lactamases (ESBLs) and other emerging beta-lactamases (BLs) have imposed concerns of the invalid usage of beta-lactam antibiotics in treating patients. The results obtained by automated instruments in clinical microbiology laboratories are often used as the primary references for the selection of therapeutic options. Rapid and accurate detection and reporting of ESBL or other BLs are clinically critical for the proper selection of antibiotics for treating patients and infection control purposes. The study was performed to compare phenotypic and genotypic methods for the detection of ESBL or other BLs in a tertiary medical center. Methods: Non-duplicate clinical isolates, including 50 *E. coli* and 29 *Klebsiella* spp. were selected with elevated MICs (>2 μg/ml) to ceftazidime, cefotaxime and aztreonam from January to April 2007. These isolates were then tested in Phoenix Microbiology System (BD Diagnostic Systems, Sparks, MD) and Vitek II System (bioMerixur, Durham, NC) for the determination of ESBL results. The ESBL confirmatory test (disk diffusion) recommended by Clinical and Laboratory Standards Institute (M100-S17, CLSI) and a modified double disk synergy test (MDDS) based on Pitout, et al. [JCM 2003, 41(8):3933-5] and French Society for Microbiology (CA-SFM 2007) were used to confirm the phenotypes of ESBL and/or other BLs. Also, two multiplex PCR for detecting predominant ESBL (TEM, SHV, CTX-M-3 & 14) and AmpC (CMY, DHA, FOX, MOX, LAT & ACC) [JCM 1998, 42(6):1350-4; JCM 2005, 43(9):4486-91; JCM 2002, 40(6):2153-62] were utilized for the determination of BL genotypes. DNA sequencing was performed to confirm TEM and SHV genotypes. Results: A total of 78 (99%) strains were tested positive for TEM, SHV, CTX-M or AmpC by PCR methods. There were 47 (59%) strains exhibited more than one type of BLs as revealed by PCR. The CLSI and MDDS methods detected 33 (42%) strains and 42 (53%) strains as ESBL positive, respectively. The Phoenix detected 55 (70%) strains as ESBL positive whereas Vitek II detected 36 (46%) as ESBL positive. None of the routine phenotypic methods (CLSI, Phoenix or Vitek II) detected AmpC or strains with multiple BLs production. Due to the production of other emerging BLs in these isolates, the phenotypic methods showed variable sensitivity and specificity in detecting ESBL. Conclusion: The study revealed that ESBL is not the only BL produced in these clinical isolates with resistance to multiple beta-lactams. Current clinical routine for the detection of ESBL may exhibit limitations on confirming ESBL and/or other emerging BLs. More specific guidelines for the detection of these BLs in clinical laboratories are warranted.

17A

Multiple PCR Combine Reverse Line Blot hybridization (RLB) to Detect Seven Sexually Transmitted Disease Urethritis pathogens

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Objectives: We have developed a multiple PCR combine reverse line blot hybridzation assay to detect STD urethritis pathogens; *Neissera gonorrheae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma genitalium*, *Mycoplasma hominis* and *Trichomonas vaginalis*. Materials and methods: Seven
primer pairs simultaneously amplified pathogen target regions. Amplified biotin-labelled PCR products, were hybridised to membrane-bound specific oligonucleotide probes. Amplified products were then inducted into pGEM-T vector and copy number of each target was calculated. The mPCR-RLB assay's clinical specimen results were compared to single PCR results.

**Results:** The assay correctly identified all reference strains and clinical specimens. There was no cross-reaction in the seven pathogens species assays. Multiple PCR-RLB can sensitively detect 10^5 copies target gene fragment. The mPCR-RLB and single PCR assay results of 211 clinical specimens were compared. It was found, only 2.8% (6 of 211) assay results were discrepant. These samples were detected by the mPCR-RLB assay, but single PCR detection was negative. Nested-PCR also detected the 6 discrepant specimens.

**Conclusions:** The mPCR-RLB hybridisation assay is sensitive and specific, and able to rapidly detect STD urethritis pathogen from clinical specimens.

**18B**

Genotyping of plasmid-mediated AmpC and randomly amplified polymorphic DNA in Klebsiella pneumoniae In Children

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**Objective:** To investigate the antimicrobial susceptibility, genotyping of plasmid-mediated AmpC beta-lactamases and the molecular epidemiology characteristic of K. pneumoniae in Children. **Methods:** The Antibiotics susceptibility of K. pneumoniae were tested by Kirby-Bauer methods. AmpC beta-lactamases was detected by triple-dimensional extract test. Genotypes of AmpC beta-lactamases were determined by multiplex PCR and DNA sequencing. And the randomly amplified polymorphic DNA technique was used in epidemic analysis of AmpC producers. **Results:** Among the 110 isolates collected, 19 strains produced AmpC beta-lactamases. The strains of AmpC beta-lactamases were highly resistant to third-generation cephalosporins, cefoxitin and antibiotics combined with inhibitors, but all sensitive to imipenem. The genotypes of AmpC beta-lactamases were DHA type. Randomly amplified polymorphic DNA analysis indicated that 19 strains have 15 types. **Conclusions:** The resistance of K. pneumoniae in Children was serious and the ratio of AmpC beta-lactamases producing strains was higher. The genotype of AmpC beta-lactamases was DHA type.

**19C**

Prevalence of aac(6’)-Ib-cr in clinical isolates of Klebsiella pneumoniae

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**Prevalence of aac(6’)-Ib-cr in clinical isolates of Klebsiella pneumoniae**

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**Abstract**

Objective: To investigate the prevalence of aac(6’)-Ib-cr in clinical isolates of Klebsiella pneumoniae. **Methods** A total of 337 isolates of Klebsiella pneumoniae were isolated from clinical specimens in our hospital from Jan, 2006 to Sep, 2007. Gentamicin-resistant isolates were screened for detecting aac(6’)-Ib-cr. The detection of aac(6’)-Ib-cr and class I interase gene (intI1) was determined by PCR. All PCR products of aac(6’)-Ib-cr were sequenced for determining aac(6’)-Ib-cr. MICs of antibiotics were determined by agar dilution method. The isolates were investigated for the presence of ESBLs by the CLSI-recommended confirmatory tests. **Results** Of the 337 clinical isolates of K. pneumoniae, 64 (19.0%), 28 (8.3%) and 55 (16.3%) isolates were resistant to gentamicin, amikacin and tobramycin, respectively. Among 64 gentamicin-resistant isolates, 24 (37.5%) were positive for aac(6’)-Ib-cr, including 13 ciprofloxacin-resistant isolates and 11 ciprofloxacin-susceptible isolates. The prevalence of aac(6’)-Ib-cr in ciprofloxacin-resistant and -susceptible isolates were 54.2% (13/24) and 27.5% (11/40). The positive rates of ESBLs and intI1 in the 24 isolates carrying aac(6’)-Ib-cr were 79.2% (19/24) and 91.7% (22/24). Plasmids carrying aac(6’)-Ib-cr of 13 isolates were successfully transferred to E. coli. Plasmids of all transconjugants were carrying aac(6’)-Ib-cr and intI1 and all transconjugants were ESBL producers. **Conclusions** aac(6’)-Ib-cr is spread widely in clinical isolates of Klebsiella pneumoniae. aac(6’)-Ib-cr and ESBL gene exist a self-transmissible conjugative plasmid by class 1 integron.

**20D**

Development of a Broadly-Reactive and Quantitative Adenovirus Real-Time PCR Assay

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**Background** Adenoviruses (Ad) are important respiratory pathogens and may cause significant morbidity and mortality among transplant patients who become systemically infected. The ability to detect Ad in respiratory specimens and to accurately quantify Ad in blood specimens is important for a modern molecular virology laboratory. Objective: We developed a new real-time assay for detection of all Ad types. We propose to use the assay as a qualitative test for detection of Ad in respiratory specimens and as a quantitative assay for measuring Ad DNA in blood of immunosuppressed patients prior to and during antiviral treatment.

**Methods** Real-time assays that targeted the Ad penton gene and hexon gene were developed separately and then combined into one broadly-reactive multiplex assay. Sequences were downloaded from GenBank and aligned, conserved regions were identified and primers and probes with appropriate Tm were chosen and tested with known isolates. The final multiplex contained two forward primers, six probes and four reverse primers and was run on an ABI 7300. We included an Internal Positive Control from Applied Biosystems, Inc. in all reactions to monitor the amplification in each well of the real-time assay. We cloned 1 Kb segments surrounding the assay target site of a representative virus from each Ad group to be used as quantitative standards and for limit of detection (LOD) calculations. Results: We used 3 separate validation panels to test the sensitivity and specificity of the Ad
multiplex assay. In Panel 1 we compared conventional culture and/or FA to the Ad multiplex assay on 26 pos and 6 neg respiratory specimens; all results were concordant. In Panel 2 we compared the EraGen PLX-RVP multiplex assay with our Ad multiplex on 33 pos and 33 neg respiratory specimens; all results were concordant. In Panel 3 we compared a published Ad multiplex (Heim et al) with our multiplex on 38 pos (32 respiratory and 6 blood) and 11 neg specimens; all results were concordant. 95% LODs were determined for representative Ad types from five species: Species A (type 31) LOD = 34; B (type 3) = 11; C (type 5) = 9; D (type 19) = 12; E (type 4) = 5 copies/rx. Quantification using standard curves made with the cloned fragments was equivalent for all 5 types from 10 to 10^10 copies of Ad DNA. Conclusions- The multiplex assay that we designed demonstrated 100% sensitivity and 100% specificity compared to (1) conventional detection of Ad, (2) a commercially available massive multiplex assay (EraGen RVP) and (3) a published real-time assay for Ad. In addition, we showed that the assay has excellent analytical sensitivity and that quantification across 5 of the 6 Ad species is similar leading us to believe that Ad of any type will be accurately quantified with this assay.

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21A
Seasonal distribution of Laribacter hongkongensis in freshwater fish in Hangzhou


Aim Laribacter hongkongensis (L. hongkongensis) was a novel genus and species associated community-acquired gastroenteritis. In this study, an investigation was launched to know seasonal distribution of L. hongkongensis in freshwater fish further.

Materials and Methods 50~80 grass carp bred artificially were dissected aseptically per month in Hangzhou (2006.11~2007.10). Fecal samples of fish were collected and transported to the lab. L. hongkongensis was isolated and identified by phenotype tests, specific primers PCR, and 16S rRNA gene sequencing. Water temperature, air temperature and pH of the fish pond were detected when fecal samples were collected. Correlation test was used to analyze correlation of air temperature, water temperature, and positive rate of L. hongkongensis isolation in grass carp. Results Samples were screened by culture media (MA and CMA), and identified by phenotypic tests and specific primers PCR. The accuracy rate of this set of methods was 100%, confirmed by 16S rRNA gene sequencing. 58 samples were positive in the study. Water temperature was highly correlated to positive rate of L. hongkongensis isolation (r=0.659, p=0.020). Change of positive rate synchronized with change of water temperature. The highest positive rate was 20.7% (12/58), as water temperature was 15°C (October 16, 2007). When water temperature was lower than 10°C, positive rate decreased to zero. Air temperature was highly correlate with water temperature (r=0.987, p=0.000). Value of pH had no correlation with the positive rate (r=-0.402, p=0.195). Discussion Air temperature was highly correlate with water temperature (r=0.987, p=0.000), and could affect L. hongkongensis seasonal distribution indirectly by changing water temperature. However, they were not consistent sometimes, for example that when air temperature was 8.9°C (water temperature 10.8°C), the positive rate was 1.67%, but when air temperature was 10.4°C (water temperature 9.6°C), the positive rate was zero. Water temperature was more suited to characterize seasonal distribution of L. hongkongensis than air temperature, and highly correlated to the positive rate (r=0.659, p=0.020). Conclusion PCR analysis was a practical method for L. hongkongensis examination. The positive rates of L. hongkongensis isolation in grass carp have obvious seasonal characteristics and decreased as the water temperature decreased. When the water temperature was under 10°C, the rate decreased to zero.

22B
Outer membrane protein GNA992 contributes to serum resistance during meningococcal sepsis

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Neisseria meningitidis is a leading cause of meningitis and septicemia worldwide with a rapid onset of disease and a high morbidity and mortality. Vaccine candidate genome-derived Neisseria antigen (GNA) 992 is a newly discovered outer membrane protein conserved among a broad range of meningococcal strains. High conservation of the protein suggests a critical role in bacterial pathophysiology. Here we study the impact of the GNA992 on meningococcal disease in vivo by using an isogenic GNA992-deficient mutant together with a mouse model of meningococcal disease. We show that GNA992 is essential for successful bacterial colonization of the nasopharyngeal mucosa. We further reveal that the protein contributes to biofilm formation and protects bacteria from being phagocytosed by macrophages, but does not affect pro-inflammatory responses. In addition, the protein confers extensive serum resistance enabling the bacteria to survive in blood during systemic dissemination and to cause lethal disease. Further study using flow cytometry analysis show that GNA992 protects bacteria from complement-mediated killing by preventing deposition of the membrane attack complex (MAC). Taken together, this study reveals a multivalent impact of GNA992 on meningococcal disease, indicating that this protein is an important target for vaccine development as well as for therapeutic treatments.

23C
Early Warning System for Foodborne Outbreaks Based on Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) of Salmonella Typhimurium and Temporospatial Clustering

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Foodborne salmonellosis caused by Salmonella Typhimurium (STM) is one of the major public health concerns in Australia and worldwide. The current challenge of STM control is the lack of strategies to focus limited public health resources onto recent outbreaks. Novel molecular typing methods appear to address this challenge by overcoming limitations of traditional
phage typing, showing better discrimination power and timeliness and allowing data exchange between laboratories and epidemiologists. The aim of this study was to implement and evaluate a system for rapid detection of STM clusters based on Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) and innovative temporal spatial clustering algorithms. Beginning in late 2006, the New South Wales (Australia) Enteric Reference Laboratory, introduced MLVA for routine typing of STM. Octal code profiles were assigned to each isolate for comparison and patients’ postal codes and dates of the disease onset captured. Temporal and spatial clustering was performed using SatScan statistic. Clonally related clusters were defined by isolates sharing indistinguishable MLVA types. A multiplex PCR-based reverse line blot assay was developed for rapid phage type identification. Near real time typing was conducted in a total of 945 isolates composed of 47 phage types over a period of 15 months. Prospective evaluation of rapid, molecular typing-based clustering identified 33-91 MLVA clusters depending on different definitions, involving 77-83% of all isolates tested. The average number of isolates per cluster was 8.6 (range 2-81) or 19.3 (range 5-81). Six endemic MLVA clusters were identified, most of which were prevalent over 10 months within the investigation period. A few major outbreaks each with more than 10 human cases were detected and differentiated from the endemic background. The majority of MLVA clustered isolates were from cases with confirmed epidemiological links and correlated with STM phage types. MLVA was highly discriminatory within the most common phage types. The specificity and the effectiveness of STM surveillance were further increased by identifying outbreaks that would otherwise have been undetected. Web-based interactive outbreak analysis tools were used to demonstrate temporospatial clustering of MLVA patterns. These methods present new opportunities to improve detection and monitoring of outbreaks of communicable diseases.

24D

A Novel Method for Rapid Diagnosis of Antimicrobial-resistant Bacterial Infections and its Application

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Background: It is important to identify antimicrobial resistant pathogens rapidly for diagnosis and treating severe infections. However, conventional methods are time-consuming, and it could not satisfy the clinical needs for bacterial identification results. It is the main reason for the low cure rate and high mortality of severe infectious diseases. Thus, we need establish a rapid and sensitive detection method. Materials and Methods: Amplify and analyze the sequence of 23S rRNA gene and the sequences of some important drug resistant genes. Based on the similarity and diversity of the sequences of these important pathogens, we designed primers and probes. Identify the bacterial species and its resistance spectrum by using microarray technique, and compare the consistency and reliability with traditional methods, validate its practicability through the application in clinical samples. Results: Using multiplex PCR and microarray technique, a new genetic method was established to differentiate common bacteria and antimicrobial resistant gene from clinical samples concomitantly. Important bacteria, including Staphylococcus aureus, coagulase-negative Staphylococcus spp., Enterococcus faecalis, Enterococcus faecium, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, Proteus spp., Enterobacter cloacae, Enterobacter aerogenes, Morganella morgani, Citrobacter freundii, Salmonella typhi, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Burkholderia cepacia, Acinetobacter baumanii, Acinetobacter lwoffii, Bacteroides fragilis, Peptostreptococcus spp., Peptostreptococcus spp., Lister spp. can be identified correctly. At the same time, SHV, CTX-M (CTX-M-1 group, CTX-M-13 group) ESBL genes, mecA, vanA, vanB could be identified. The detection system were evaluated with convention methods by clinical isolates, drug-resistant standard strain, and it appeared to be a sensitive, specific, and reproducible tool for detection or identify common antimicrobial resistant bacteria. 173 blood samples, 339 cerebrospinal fluid, pleural fluid, ascitic fluid samples, 514 urine samples were collected. The results using genetic method of 97.8% blood samples, 99.8% cerebrospinal fluid, pleural fluid, ascitic fluid samples, and 99.7% urine samples, totally 99.3% samples were consistent with that of traditional method. Using genetic method detection we can get the result 2.0±1.15 days before the traditional method detection.

Conclusion: Multiplex PCR-microarray methods can be used in the etiologic diagnosis of drug resistant bacterial infections. Using this technique would improve the prognosis of patients.

25A

Microbial Nucleotide Target Detection and Identification by Mass Spectrometry

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In clinical laboratories the framework of molecular typing is comparative sequence analysis by dideoxy sequencing in large collections of microbial isolates utilizing molecular marker regions. New informative molecular marker sets for the identification and characterization have been developed and are still needed to differentiate amongst and between pathogenic and non-pathogenic strains. We have recently developed new algorithms and software tools for automated, high-throughput comparative sequence analysis by MALDI-TOF mass spectrometry. Mass spectra are acquired after PCR, in vitro transcription and base-specific cleavage of 500-800 bp target regions. A reference sequence or a set of reference sequences is used to simulate cleavage patterns in silico, which serve as a comparative measure for the cross-correlation of theoretical and experimental mass signal patterns. In theory, samples can be identified by finding the best match of the detected peak pattern with the simulated pattern of the references, but missing and additional peaks due to deviations between the sample and the best match, contaminating adduct peaks, intensity variations and the overall spectra quality required the implementation of an iterative identification process and the development of a quality scoring scheme. Using these new algorithmic approaches, we developed analysis routines reporting on sequence identification results, confidence levels and identified microheterogeneities between the best matching reference and the sample reference to deliver new reference sequences. 32 marker regions of potential importance for the development
of a new molecular typing scheme for N. gonorrhoeae and a set of 267 phenotypically characterized samples were successfully analyzed with this method. Marker and N. gonorrhoeae sample identification were a result of UPGMA peak pattern clustering, statistical analysis and sequence analysis by a time-efficient SNP Discovery algorithm. Dideoxy sequencing for samples of high variance showed concordance with the mass spectrometry data. Applications of the presented biochemistry in combination with MALDI-TOF mass spectrometry include species and sub-species identification as well as molecular resistance typing and comparative sequence analysis of haploid organisms in general to enhance current research and clinical approaches as well as microbial outbreak monitoring and clinical management.

26B

Rapid Diagnosis of Mycobacterium Tuberculosis with p-nitrobenzoic acid by the MGIT™ 960 System

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Background: With the re-emergence of tuberculosis in different regions of the world, precise and time-saving diagnosis for Mycobacterium tuberculosis (MTB) has once again drew forth attention. Growth of MTB is sensitive by adding inhibitory substances p-nitrobenzoic acid (PNB) into culture media; whereas Non-tuberculous mycobacterium (NTM) is resistant. The study was performed to compare the effectiveness and reporting time of conventional biochemical reactions and PNB differentiation test of the MGIT 960 system (Becton Dickinson Diagnostic Systems, Sparks, MD). Methods: A total of 116 mycobacterial isolates, composed of 33 NTM strains and 83 MTB strains identified by conventional biochemical tests were selected for the evaluation. Isolates were inoculated into BACTEC MGIT 960 liquid media with PNB-containing and PNB-free control tubes. Results of susceptibility and time to result (TTR) were recorded. Results: The 83 MTB isolates are all susceptible to PNB/PNB method. Among 33 NTM species, all are resistant except for one Mycobacterium kansasi strain. The sensitivity and specificity of the MGIT/PNB method to differentiate MTB from NTM strains was 100% and 97%. The median TTR of presumptive identification of MTB in the BACTEC MGIT 960 system was 9 days and for NTM it was 5 days. Conclusions: PNB differentiation test combined with liquid media culture system is a simple and efficient method to differentiate MTB from NTM. With the faster TTR, it could be helpful to ease epidemic tuberculosis by earlier diagnosis.

27C

The effect of inducing ampC of Pseudomonas aeruginosa biofilms exposed to β-lactam antibiotics

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Objective To study the inducing abilities of various β-lactam antibiotics for ampC mRNA expression in Pseudomonas aeruginosa (PA) biofilms and to explore the possible cause of PA resistance. Methods PA01 Biofilm was established in a silicone tube after incubation 72h, and then exposed to several β-lactam antibiotics for 7h, with concentrations equal to minimal inhibitory concentrations (MIC). The bacteria cells in biofilms were collected, then total RNA was extracted and reverse transcripted to cDNA. The mRNAs expression of ampC were compared by semi-quantitative PCR and some of them were compared by real-time PCR. Results The relative optical density of ampC mRNA of PA biofilms exposed to common antibiotics including imipenem, meropenem, panipenem, ceftazidime, cefepime and piperacillin is 4.4±0.13, 3.5±0.4, 2.1±0.07, 1.2±0.11, 1.52±0.15 and 1.1±0.03. Carbopenems antibiotics including imipenem, meropenem, panipenem higher than negative control with no exposure to antibiotics(P<0.001). The copies of ampC mRNA of PA biofilms exposed to antibiotics including meropenem, panipenem, ceftazidime, piperacillin and two negative controls were 5.62, 6.01, 5.48, 4.56, 5.53, 5.82. Conclusion Carbopenems antibiotics including imipenem, meropenem and panipenem can induce the ampC mRNA expression of PA, while penicillins and cephalosporins don’t exhibit induction effect of ampC mRNA expression. Bacteria in PA biofilms might develop resistance under the exposure to inhibitory concentrations of carbopenems.

28D

Evaluation the practicability of Cord formation of MGIT positive smears for Presumptive Identification of Mycobacterium tuberculosis

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Background: More than 50% Mycobacterium isolates are Non-tuberculous Mycobacterium (NTM) in most mycobacterial laboratories in Taiwan. Species identification of mycobacterium requires the use of time-consuming conventional biochemical methods, or more rapid molecular techniques that are expensive and require special instrument. Cord factor is known of the specific toxic factor of Mycobacterium tuberculosis (MTB). Most of MTB can be examined the cord formation in acid fast staining smears (AFS) of direct specimens or liquid cultures, and on young culture colonies of Middlebrook 7H10 or 7H11. The aim of this study is to evaluate the practicability of microscopic examination of the cord formation in BACTEC MGIT (Becton Dickinson Diagnostic Systems, Sparks, MD) culture positive smears for presumptive identification of MTB. Method: A total 522 acid fast positive smears of MGIT cultures were collected on October 2007. Each of smears was examined the microscopic morphology, including cord, pseudo-cord, needle, dot, short, ladder. Species identification of all mycobacterium isolates were undertaken by conventional biochemical techniques. The sensitivity and specificity of cord formation for discriminating MTB from NTM were calculated by using conventional biochemical techniques as standard. Result: Of 522 MGIT-positive cultures, 250 were identified as MTB and one mixed with MTB and M. avium complex(MAC) by conventional method. The remaining 271 cultures were NTM, including 112 MAC, 21 M. kansasii, 39 M. abscessus, 18 M. fortuitum group, 7 M. gordonae, 6 M. scrofulaceum, 2 M. mucogenicum, 3 M. marinum, 38 unidentified NTM, 3 mixed with two NTM species, 2 mixed with MAC and unidentified NTM. Of 251 MTB isolates, 249 were found cord formation, including the MTB and MAC mixed culture. Of 271 NTM cultures, only one was found cord formation, and 21 were found
pseudo-cord. The overall sensitivity and specificity of the cord formation was calculated to be 99.2% and 99.6%.

**Conclusion:**
Our results manifested that the usefulness of the cording morphology as a rapid and simple microscopic examination test for presumptive identification of MTB in MGIT-positive cultures with high sensitivity and high specificity. Because pseudo-cord might be confounded as the cord formation of MTB, examiner must have high experience for microscopic morphologies of mycobacteria. We suggest that the cord formation can only be employed as a rapid screening examination for MTB. It still required further confirmation by other test techniques.

**29A**

A new enzyme immunoassay based on hepatitis C virus envelope antigen E2 specific-binding peptides developed for the early detection of HCV infection

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**Backgrounds:** Currently the diagnosis of Hepatitis C virus (HCV) infection is mainly based on the detection of anti-HCV Abs by ELISA or on the HCV RNA detection by RT-PCR. However these methods are either lack of detecting early infection due to the so-called window period, or highly expensive and not suitable for large population screening. HCV core antigen is the only one developed, recently, for the detection of viral antigen in some developed countries. However the detection for more HCV structural antigens as new diagnosis kits for the early detection still remains a high priority target. Objective: To develop a new enzyme immunoassay for the HCV envelope structural antigen E2 to improve the early detection of HCV Infection.

**Methods:** The selected peptides which specifically bound with HCV envelope E2 protein and HCVcc with high affinities were obtained by the ribosome display technique. Different selected peptides bind with different sites of E2. And these selected E2-binding peptides were also evaluated to bind to six types (1a, 1b, 2a, 3, 4, 5, and 6) of HCV E2. Basically the procedures of this new enzyme immunoassay ELISA were described as following: E2 antibodies were pre-coated on the plates, and sera samples were added and subsequently incubated with the biotin-labeled E2-specific binding peptides. HCV were detected using HRP-labeled streptavidin and colors were developed by adding substrate.

**Results:** In order to evaluate whether this new enzyme immunoassay is highly specific for the detection of the early HCV Infection, 180 samples from body check donor samples (which HCV antibody is negative) were measured, and results showed that more than 96% of the samples were below the cutoff values and defined as HCV negative by the use of this method., only 5 sera samples (2.8%) were detected as HCV positive in 180 samples which might be in the so-called window period. Furthermore, another 25 sera samples from only HBV positive patients were all shown as HCV negative by this ELISA method. To further evaluate whether this new enzyme immunoassay is highly consistent with HCV RNA RT-PCR detection method, 85 HCV patients sera samples (which HCV RNA is positive: 10^4~10^7 copies/ml) were detected as 100% positive by the use of this ELISA method., which illustrates that this method is highly specific and consistent with HCV RNA RT-PCR detection method.

**Conclusions:** The new enzyme immunoassay based on HCV E2 specific-binding peptides presented here for the early detection of HCV infection could be a useful alternative to HCV RNA detection or HCV core Ag assays for diagnosis or blood screening when nucleic acid technologies or HCV core Ag detection are not implemented. This ELISA method could also be further developed to evaluate the mutagenesis of E2 in HCV patients.

**30B**

An Integrated PCR-Microarray Chip for the Detection, Differentiation and Identification of Influenza Viruses including H5N1

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Current fears over the threat of a possible avian flu pandemic necessitate the rapid detection and identification of influenza viruses. Here, we describe the utility of an integrated PCR-microarray platform, the ST's In-Check™ Lab-on-chip, which could enhance influenza virus surveillance efforts. This platform is based on a miniaturized silicon chip that integrates a PCR reactor with a customizable low-density DNA microarray. This Lab-on-chip allows multiplex RT-PCR followed by hybridization of RT-PCR products to a low-density DNA microarray to be performed on-chip. The probes of the microarray were designed to identify clinically relevant subtypes of influenza A, including H5N1 as well as influenza B in one single test. Also, included on the PCR-microarray chip were probes that could potentially allow the detection of mutations associated with resistance to neuraminidase inhibitors. In this study, 20 clinical isolates of influenza were correctly identified as H3N2, H1N1 subtypes and as influenza B isolates. No cross reactivities of the probes were detected when non-influenza respiratory viruses such as parainfluenza, rhinovirus and human coronavirus (229E and OC43) were tested. The Lab-on-chip diagnostic approach could facilitate the rapid detection and characterization of circulating influenza subtypes.

**31C**

Phylogenetic Relationships of Rhinoviruses Detected in Patients with Severe Acute Respiratory Disease

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Human rhinoviruses (HRVs) are increasingly recognized as agents of severe respiratory disease. Recently, apparently unculurable (HRVs) were recognized that do not belong to the two recognized genetic subgroups, and in one study were associated with influenza-like illness. As part of a study of severe acute respiratory disease, we tested bronchoalveolar lavage (BAL) specimens from severely ill hospitalized patients with the Eragen MultiCode-Pix Respiratory Panel, a multiplexed PCR-based assay capable of detecting 17 respiratory virus targets. 72 respiratory virus specimens were detected in the 485 BAL specimens.
32 of 72 respiratory viruses detected were identified as HRVs, making it the most common group of respiratory viruses detected. Of 32 specimens positive for HRVs by the Eragen assay, 24 had viral isolation performed, of which only 5 were detected by culture, underscoring the significant increase in detection of HRVs afforded by molecular methods. Detection of HRVs from cases of severe disease could be explained by the existence of highly pathogenic strains. To determine whether the viruses detected from these patients were associated with a particular phylogenetic group we performed sequencing of two different regions of the HRV genome and compared the results to those of all 102 reference serotypes. Initially, HRV positive specimens were amplified with a set of primers targeting the VP4/VP2 region. Of the 25 specimens yielding sequence data, 17 sequences clustered with A clade viruses, 4 with a newly reported novel group, 3 with B clade viruses, and 1 with HRV 87/enterovirus 68. This distribution was similar to that of 15 HRVs detected in specimens submitted for routine viral studies and not selected for patients with severe respiratory disease. A segment of the 5’ noncoding region (NCR) was also sequenced and in all cases NCR sequences confirmed the VP4/VP2 sequencing results. In conclusion, using a molecular method of detection, HRVs were the most common respiratory viruses detected in BAL specimens from a large group of hospitalized patients with severe acute respiratory infection. Sequencing results from two regions of the HRV genome indicated that the viruses detected were distributed across both known genetic groups of HRVs and also included members of a newly recognized branch of clade A that did not cluster with any of the known HRV serotypes. Isolates from the severely ill patients had a similar distribution to those of a group of patients not selected for severe illness. Grant support: Midwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Grant # 5U54 AI057160.

32D

Comment on etiological relationships between Reovirus and Severe acute respiratory syndrome (SARA)

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Severe acute respiratory syndrome (SARS) emerged in China and other countries during 2002-2003. Since World Healthy Organization (WHO) announced that SARS Coronavirus (SARS-CoV) as the etiologic agent of SARS. SARS-CoV was studied deep by experts. However, at least two important issues almost have not been resolved drastically. One, SARS was caused by single or multiple etiologic agents. Second, natural hosts of agents of SARA were known unclearly. Base on some reports, The novel Reoviruse (ReoV) were isolated from Chinese patients of SARS during outbreak of SARS in China. The antibodies against ReoV and SARS-CoV were detected simultaneously in double serum from patients of SARS. ReoV and SARS-CoV were isolated simultaneously from a patient of SARS by Laboratory of Beijing 302 Hospital that was supported by electron microscopical examination. In animal virulence pathology, pathogenic of cavy lung infected by isolated ReoV or two novel viruses, ReoV and SARS-CoV, resembled that of clinical patients of SARS. In conclusion, novel ReoV and SARS-CoV may have etiological relationships of co-infection in some patients of SARS. While the reservoir hosts were investigated also. Recent studies have suggested that bats are the natural reservoir of SARS-like CoVs. A case of acute respiratory tract illness might be associated with bat in Melaka, Malaysia. A 39-year-old male patient (MRA) has not been involved in field activity for the last 2 years and his family did not keep any pets. However, 1 week before the onset of his fever, a bat flew into his living room, while he was watching television. The bat flew “frantically” and flew out. On March 20, 2006, MRA had symptoms of high fever, cough and sore throat. About 1 week after the onset of his fever, his two children (daughter and son) developed a high fever. A virus was isolated named Melaka Virus (MeV) from MRA. Genome sequence analysis indicated a close genetic relationship between MeV and Pulau Virus (PulV), a Reovirus isolated in 1999 from fruit bats in Tioman Island, Malaysia. The serum from MRA and his family members were tested for the presence of antibody against MeV. Four members were positive. These data indicate that MeV is bat-borne Reovirus and capable of infecting and causing disease in humans. The infection of multiple members in the same family suggests human-to-human transmission. The SARS-like CoVs and MeV are bat-borne viruses and some cases were co-infected by SARS-CoV and novel ReoV during outbreak SARS in Chine show that SARS-CoV and novel Reovirus possible have the same reservoir host, bats. The agents spillover from bats enhance the risk of genetic diversity. The host shifts could increase lethal ability and may indicate a risk of novel disease emergence. These requirements support the need for further research on relationships between bats and emergence of SARS.

33A

Identification of scrub typhus, spotted fever group and typhus group rickettsioses by duplex and nested PCR

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Recent years, new species and prevalences have been identified continually, so Rickettsiae diseases have been attented worldly. Because of unspecific clinic features, PCR method already be used to diagnose Rickettsia universally. This study try to establish a duplex and nested PCR methods that can identify multi-members of genera of rickettsiae and genera of O.tsutsugamushi simultaneity and investigate the natrual foc of rickettsioses in Hongta areas in Yuxi city, Yunnan provence. Methods: Outer primers were designed based on the conserved regions of heat shock protein groEL gene of genera Rickettsiae and genera of O.tsutsugamushi and two sets of inner primers targeting to
genera of *Rickettsia* and genera of *O. tsutsugamushi* respectively were derived from literature (Hyo-Soon Park, 2005). The genera, species and type of *rickettsiae* were differentiated by different sizes of PCR products and sequencing or PCR-RFLP. The 354 mouse viscera samples including liver, spleen and kidney, and 96 Xenopsylla cheopis and 32 chiggers were collected from Hongta areas of Yuxi city, Yunan province and then the extracted DNA were tested by the established PCR methods. **Results:** After the nested PCR, 217-bp DNA fragments were amplified from the members of genera *Rickettsia* include the typhus and spotted fever group rickettsiae and 364-bp DNA fragments were amplified from members of genera *O.tsutsugamushi*. All rickettsial strains of genera *Rickettsia* and *Orientia tsutsugamushi* in this study were obtained positive amplification and the PCR fragments were further confirmed by the sequence analysis. The sensitivity of the methods reached at one copy of genera *Rickettsia* and *Orientia tsutsugamushi* rickettsial plasmids. The digestion with restriction endonuclease *RsaI* and *HinfI* could easily distinguished among the common stains of *O. tsutsugamushi*. Typhus group rickettsiae were distinguished from spotted fever group rickettsiae by digestion with *RsaI*. The results of investigation showed that the total infection rates of mouse was 34.78% (120/345), the infectious positive rates of *R. rickettsii*, *O. Karp* and *R. sibirica* were 28.12% (97/345), 19.71% (68/345) and the 0.29% (1/345) respectively, the coinfection rates of *R. rickettsii* and *O. Karp* was 13.33% (46/345). The diagnosis was supported by the detection of vectors samples including *Xenopsylla* cheopis and chiggers collected from mouse. The presumed animo acid sequences of some isolates existed variation of 1% to 2%. The *O.t* Karp type was the main epidemic train in this areas. **Conclusions:** This PCR method could be widely used to detect multi-genera *rickettsiae* quickly and it suit diagnose of Epidemic typhus, Murine typhus, Spotted fever and Scrub typhus simultaneously. Molecular evidences in this study and previously research strong indicate that Hongta areas of Yuxi city are natrual focs for typhus and scrub typhus and coinfection of them are very common.

34B

Epidemiological and molecular analysis of nosocomial Acinetobacter baumannii infections

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The heterogeneous group of bacteria belonging to the genus *Acinetobacter* is frequently implicated in opportunistic infections of critically ill patients in hospitals. Nosocomial *Acinetobacter* spp. infections are frequently attributed to *A. baumannii*, gen.sp. 3 and gen.sp. 13TU, all belonging to the so-called *A. baumannii-A. baumannii* complex. *A. baumannii* is gradually gaining more attention as a public health threat because once on a ward, it can easily spread between patients and persist in the environment. In addition, *A. baumannii* nosocomial isolates often are multidrug-resistant (MDR). *A. baumannii* MDR isolates from geographically distant areas can be clonally related, suggesting that resistance might be associated with a limited number of successful lineages. As a consequence of the above-reported considerations, the management of *A. baumannii* infections has become a serious problem in many countries. Epidemiological typing is important for outbreak investigation, allowing determination of clonal spread in a microenvironment and identification of the source and the mode of infection transmission.

In this study, strains of *A. baumannii*, isolated from different wards of Florence hospitals, were typed by the Amplified Fragment Length Polymorphism (AFLP) technique to investigate their genetic relatedness. Most of isolates 78% belong to two clonal groups, A1 and A2, that spread across time and space; the other strains do not belong to any clonal complex. As regard the A1 e A2 clones: 1) retrospective studies revealed that these clones were already isolated from infected patients recovered two years before in the same wards; 2) clonal group A2 was responsible for an outbreak and re-appeared two months later in the same ward, confirming the persistence of this clone in hospital settings; 3) clonal group A1 is widely spread in almost all the wards of one hospital; epidemiological investigation revealed that its presence in a ward of a different hospital could be explained by the transfer of a colonised patient. AFLP typing demonstrated to be an important tool in *A. baumannii* infection prevention and control and it could be used to investigate the genetic determinants underlying their antibiotic resistance.

35C

Bacteriological Profile of Urinary Tract Infections in Patients Attaining Nepal Medical College Teaching Hospital, Kathmandu

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**Background:** This paper reports the urinary pathogens and their antibiotic patterns. **Methods:** A total of 1079 ‘clean caught’ midstream urine samples received from the inpatients and outpatient departments of Nepal Medical College Teaching Hospital from April 25, 2006 to March 3, 2007 were included. Firstly, samples were subjected for culture using standard microbiological procedures then examined microscopically for pus cells and casts. Culture was done on the blood agar (BA) and MacConkey agar (MA) medium by standard calibrated loop and incubated at 37°C overnight. Culture results were interpreted as being significant (≥10⁵ colony forming units/ml) and insignificant, according to the standard criteria. An antibiotic susceptibility test for bacterial isolates was done by Kirby-Bauer technique. **Results:** Significant growth positive was 25.6% (276/1079). *Escherichia coli* was the commonest isolates (77.5%) followed by *Klebsiella* spp (11.6%), *Proteus mirabilis* (7.6%), *Pseudomonas aeruginosa* (2.5%) and *Staphylococcus sp.* (0.7%). Amikacin was the most effective (99.2%) and was followed by tobramycin (96.5%), nitrofurantoin (86.7%) cefotaxime (85.7%) and ciprofloxacin (78.0%), all the pathogens isolated. *E. coli* was most sensitive to amikacin (96.8%), followed by tobramycin (96.1%), nitrofurantoin (81.7%), cefotaxime (80.3%), ciprofloxacin and ofloxacin (76.6%), nalidixic acid (52%), norfloxacin (49.2%) cephalaxin (38.5) and cotrimoxazole (29.0%). **Conclusion:** Data presented in this study indicate that antibiotics commonly used for the treatment of UTIs are less effective. Since this was a cross-sectional study, further regular monitoring is required to establish reliable information about resistance pattern of
36D

Identification of Mycobacterium Tuberculosis and Other Species of Nontuberculous Mycobacteria by Real-Time Per and Melt Curve Analysis


Introduction/Background: Nontuberculous mycobacteria (NTM) cause serious illnesses in diverse patient populations. Consequently, the rapid identification of all mycobacterial species is extremely important. Conventional identification and detection methods for mycobacteria are time consuming and may not differentiate closely-related species. A real-time PCR technique was developed that identifies 19 species of mycobacteria directly from positive culture broth in less than six hours.

Materials and Methods: Two real-time PCR assays using consensus primers and fluorescent probes for the hsp65 and the 16S rRNA gene sequences were developed using mycobacterial reference strains. These assays were clinically validated using 107 clinical samples. Genomic DNA was extracted from Mycobacterial Growth Indicator Tube (MGIT) broth using a simple boil and centrifugation lysis protocol; the supernatant was used for real-time PCR. Real-time PCR assays for the IS6110 repeat sequence and a previously published 16S rRNA gene (Shrestha et al., 2003) were used for more complete identification of certain species.

Results: The hsp65 PCR/melt curve assay alone correctly identified four individual species (M. kansasi, M. abscessus, M. szulgai, M. simiae) and six pairs or triplet groups (M. chelonei/terae, M. fortuitum/marinum, M. antibioticus, M. xenopi, M. mucogenicum/haemophilum, M. avium/flavescens, and M. intracellularure/scofulaceum/tuberculosis complex). Individual species in five of these six groups were singly identified using the new 16S rRNA assay. The final group consisting of M. intracellularure/scofulaceum/tuberculosis complex can be separated using the previous Shrestha 16S rRNA and IS6110 PCR assays, resulting in complete identification to species, with 80/81 (99%) of the clinical isolates identified directly from MGIT broths. Additionally, all 26 MGIT broths with no growth or with growth other than mycobacteria were negative via the hsp65 assay.

Conclusions: Application of these real-time PCR assays with melt curve analysis provides a rapid and accurate method for the identification of mycobacteria, including M. tuberculosis, directly from positive culture broth.

37A

High Incidence of Campylobacter Contamination in the Lake Kochunam

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Intestinal pathogenic bacteria, Campylobacter, were detected in water samples collected from the Lake Kochunam, South Korea. PCR detection was performed after enriching the 50ml of water samples and MPN-PCR method was applied to quantify the amount of Campylobacter. The source of Campylobacter would be migrating geese from Siberia through China. More than 10,000 of migrating geese land in the Lake Kochunam every winter. Another possible source would be livestock animals as the Lake Kochunam is surrounded with paddy fields and small livestock farms, so it is possible to think that rain runoff have brought pathogens into the lake. Water in the lake is mainly used for irrigation in the area, by which we human may intake the pathogenic bacteria through agricultural crops produced in that area. We report the epidemiological potential public health risk most likely caused by the migrating geese.

38B

Use of Real-time PCR for the Detection and Identification of Rickettsiae in Clinical Diagnosis, and Research and Surveillance Studies

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Rickettsial diseases include the typhus group rickettsioses consisting of epidemic typhus (Rickettsia prowazekii) and murine typhus (Rickettsia typhi), the spotted fever group rickettsioses such as Rocky Mountain spotted fever (Rickettsia rickettsii), Mediterranean spotted fever (Rickettsia conorii), flea-borne spotted fever (Rickettsia felis), and scrub typhus (Orientia tsutsugamushi). These diseases have been and continue to be a risk of morbidity and mortality to people throughout the world. Rickettsioses can be diagnosed by the detection of the etiological agent using real-time polymerase chain reaction (PCR) assays at the time of acute illness. In addition, the real-time PCR assays can be used to detect genus, group and species specific rickettsiae in vectors and/or their hosts in research and surveillance studies. Due to their extreme sensitivity and specificity these assays developed in our laboratory have successfully detected the rickettsial agents R. felis, R. parkeri, and R. bonei TT118 in clinical samples (whole blood,uffy coats, sera, and biopsies of rash and eschar), O. tsutsugamushi in research samples (blood and other tissues from monkeys and mice), and R. prowazekii, R. typhi, R. felis, R. amblyommi, R. montanensis, and O. tsutsugamushi in surveillance studies (blood from peridomestic rodents, ticks, lice, mites and fleas). The high specificity, sensitivity and reproducibility of these assays make them among the best emerging technologies for the diagnosis of infectious diseases and in the detection of pathogenic organisms.

39C

Development of a Real-time PCR Assay for the Detection of Orientia tsutsugamushi and Its Use in Evaluation of Blood Samples from a Scrub Typhus Vaccine Study Employing a Non-human Primate Model

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Scrub typhus, a mild to fatal febrile illness of humans, is caused by the bite of chiggers infected with the etiologic agent, Orientia tsutsugamushi, and is endemic throughout the Asia-Australia-Western Pacific Islands region. Previously our laboratory demonstrated that a quantitative real-time PCR (qPCR) assay developed to diagnosis scrub typhus by targeting the conserved outer membrane 47 kDa protein gene was both sensitive and
specific in recognizing *O. tsutsugamushi* DNA. Because this same r47 kDa protein gene has been successfully utilized as a vaccine candidate a new qPCR assay targeting another gene was produced. This report describes the development and use of a new scrub typhus qPCR assay in detecting a 145 bp fragment of the conserved 22 kDa protein gene of *O. tsutsugamushi*. The assay using a molecular beacon probe was found to be sensitive (detects as low as three DNA copies per reaction) and specific (recognizes 21 out of 22 strains of *O. tsutsugamushi* DNA but not rickettsial, non-rickettsia bacterial, human, mouse or monkey DNA). In a non-human primate scrub typhus vaccine study, this assay was used in the evaluation of blood samples from 20 cynomolgus monkeys collected every other day during a 30 day period following challenge. Evidence of bacteremia was detected as early as day 7 (range 7 - 9) in the control monkeys and as early as day 12 (range 12 - 16) in the vaccinated monkeys. The data described herein suggests that this new scrub typhus qPCR assay based upon the 22 kDa antigen gene target has the potential to be very useful in the detection of *O. tsutsugamushi* in clinical and research samples.

40D

Title unavailable

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Molecular methods, for identification and strain typing of microbial pathogens, are becoming increasingly important in clinical microbiology. Methods such as PCR/sequencing of suitable targets or whole DNA fingerprinting methods, such as PFGE, are often seen as “gold standards” for molecular identification or genotyping, respectively, but unsuitable for routine use. Faster, more convenient methods are being developed but the choice depends on target genera/species, resources and indications for testing. Based on our experience, in a large diagnostic/public health microbiology laboratory, we compare the use of, and indications for, 3 relatively new methods - reverse line blot (RLB) hybridisation assays, rolling circle amplification (RCA) and high resolution melt curve analysis (HRMA). RLB hybridisation of multiplex or single PCR amplicons can be used to simultaneously detect presence/absence (binary typing) of >40 targets in a “macroarray” format, using suitably designed probes. We have used this method to identify a) >20 potential pathogens in clinical specimens e.g. genital, respiratory; b) species within microbial groups or genera e.g. mollicutes, yeasts, *Mycobacterium* spp; c) serotypes e.g. of *Streptococcus agalactiae*, *S. pneumoniae*, d) genotypes and multiple antibiotic resistance or virulence markers e.g. in MRSA, *E. coli*. It can be applied directly to specimens for culture-independent identification or genotyping but takes ~16 hours, which makes it less suitable for diagnosis than real-time methods. RCA is a highly specific, very rapid isothermal amplification system, suitable for diagnosis or genotyping using a limited number of targets. It can be used to identify highly conserved species- or strain-specific single nucleotide polymorphisms (SNPs) at single or multiple (up to 12) sites, simultaneously e.g. to distinguish *S. agalactiae* III subtypes, dermatophyte species, *Cryptococcus* subspecies, MRSA *femA* sequence types (which correspond with clonal clusters) and *Mycobacterium tuberculosis* or HIV drug resistance mutations. HRMA is a real-time PCR-based method that can distinguish gene alleles based on amplicon melting temperatures. We have used it to distinguish *Ureaplasma* species, *C. trachomatis* LGV serotypes and various *M. tuberculosis* resistance gene mutants. All of these methods are relatively inexpensive, objective and simple, with sensitivities and specificities comparable with those of gold standard methods, if used for appropriate indications.

41A

An aptamer from whole-bacterium SELEX as a new diagnostic tool binds tightly and selectively to *Mycobacterium tuberculosis*

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Targeted testing and treatment of individual with latent tuberculosis infection at increased risk of progression to active disease is a key element of tuberculosis control. This strategy is limited by the poor specificity of the tuberculin skin test in populations vaccinated with bacille Calmette-Guérin (BCG) and its low sensitivity in immunosuppressed persons, who are highest risk of progression. To develop high-affinity ligands capable of selectively recognizing *Mycobacterium tuberculosis* as putative diagnostic tools, we applied a whole-bacteria Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technique and identified a ssDNA aptamer NK2 that binds to virulent strain *Mycobacterium tuberculosis* (H37Rv) with high affinity and specificity. The *K*a value between NK2 and H37Rv was measured by ITC (Isothermal titration calorimetry) and determined to be 1.84×10^{-6}±(1.5×10^{-6}) Lmol^{-1}, or 2.85×10^{-5}±(6.0×10^{-6}) Lmol^{-1}. We found that the aptamer NK2 strongly inhibited H37Rv from adhering to or invading macrophages and stimulated the intracellular IFN-γ production of CD4+T cells *in vitro*. The survival rates of mice challenged with H37Rv have been prolonged after treatment with single injection of aptamer NK2. The bacterial numbers significantly declined in the spleen of mice treated with aptamer NK2. The histopathological examination of lung biopsy specimens showed lesser pulmonary alveolar fusion and swelling in the presence of the aptamer. Further we will evaluate the feasibility and validity of NK2 as a diagnosis reagent in clinical practice. The selected ssDNA aptamer NK2 binding with high selectivity to *Mycobacterium tuberculosis* hold strong potential both as a new molecular probe or a diagnosis reagent and a tool to analyse the interactions of *Mycobacterium tuberculosis* and host cells.

42B

XF-73: A New Approach for Nasal *Staphylococcus* Decolonisation: No Emergence of Resistance in Multiple MRSA Strains, After 55 Passages


Background: Nasal carriage of *Staphylococcus aureus* is associated with an increased risk of infection. Nasal decolonization is an integral part of infection control protocols. The development of rapid screening diagnostics will lead to greater demand for effective decolonization products. A key issue with topical antibiotics such as mupirocin is the emergence of resistance - effective decolonization being severely compromised. XF-73
is a novel antibacterial agent which has previously been shown to be rapidly bactericidal (unlike mupirocin, which is bacteriostatic) and to possess a long post-antibiotic effect. A study is presented where XF-73 and mupirocin are compared for emergence of resistance in a number of methicillin-resistant *Staphylococcus aureus* (MRSA) strains, investigated through extended multiple passage exposures. **Methods:** Minimum inhibitory concentrations (MICs) were determined for both XF-73 and mupirocin using a macrodilution broth method (CLSI formerly NCCLS; Document M7-A7, Volume 26 No.2 January 2006: Approved Standard - Seventh Edition). Five “Network on Antimicrobial Resistance in *Staphylococcus aureus*’’ MRSA strains were tested: NRS382 (USA100, a healthcare-associated strain), NRS384 (USA300, the predominate US community-associated strain), NRS271 (linezolid-resistant, containing phage type E-MRSA 15), NRS123 (USA400, a multiply-susceptible community-associated strain which carries the Panton-Valentine leukocidin genes and additional virulence genes distinct from hospital-associated MRSA strains) and NRS387 (USA800, also known as the Paediatric clone). All 5 strains were passaged 55 times at 0.5x the MIC (determined from the previous passage) to investigate if there was an increase in the MIC. An increase in the MIC would suggest the development of resistance. **Results:** The initial MICs of XF-73 and mupirocin were 0.25 - 0.5 mg/L and 0.12 - 0.25 mg/L, respectively against the 5 MRSA strains tested. For all 5 strains, a significant increase (> 8 fold compared with original) in the MIC of mupirocin was observed after only 2 - 7 repeat passages, with the MIC reaching 8 - 512 mg/L after 55 passages. The largest increase observed was in the USA300 community-associated strain. In comparison, the MIC of XF-73 did not increase significantly for any of the 5 MRSA strains, even after 55 passages. **Conclusions:** All 5 MRSA strains developed resistance to mupirocin after only a few repeat passage exposures. In comparison, XF-73 showed no significant increase in MIC against the same MRSA strains, even after 55 repeat passages. This suggests that the likelihood for resistance development against XF-73 is remote. Therefore XF-73 may open up new routes for widespread infection control, which are currently closed to antibiotics, and may represent a new pharmaceutical approach in the fight against bacterial infection which complements the new era of rapid diagnostics.

**43C**

The Impact of a PCR-based Diagnosis for Enteroviral Meningitis in a Large Pediatric Hospital.

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PCR-based laboratory diagnosis of Enterovirus meningitis was instituted in July 2007 using the Cepheid FDA-approved Enterovirus Assay for the GenXpert platform. A retrospective comparison between July and October 2006, when culture was the primary diagnostic technique, to the same period in 2007 was made. The goal of the study was to evaluate the impact of the PCR assay on the duration of the patient’s hospital stay and the number of days of antibiotic treatment. Each positive case was examined for the initial diagnosis, number of hospitalization days along with the duration and total cost of antibiotic use. Only 6 Enterolike-virus positive viral culture results were identified from July 06 to October 06; the total number of enterovirus cultures could not be determined because a specific enterovirus culture did not exist. In 2007, 16 positive EV-PCR-CSF from 65 patients (25%) results were found. Before the initiation of the PCR-based enterovirus test, the median duration of hospital stay was 4 (range 3-7days) days and the median antibiotic treatment lasted 3 (range 0-3) days. In the corresponding months in 2007, the median hospitalization time remained unchanged at 4 (range 1-6 days) with a reduction in antibiotic treatment days to 2 (range 0-3) days. The Cepheid assay requires 3 hours of PCR-time and by agreement with the Pediatric Emergency Room, PCR-tests were done once per day. The result turn-around-time for culture was 4-5 days. The overall impact of this easy to perform PCR assay on length of stay and cost may be improved in the next year by performing the assay while the patient is in the Emergency room.

**44D**

Bactericidal and Fungicidal Activity of Different Honeys

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**Background** With the irrational and massive use of Antibiotics in underdeveloped and developing countries, resulantly there was increased Resistance to Antibiotics and with the increased interest in honey for various therapeutic purposes has led to the search for new antibacterial honeys. Hence a study was conducted to assess the antibacterial and fungicidal activity of five locally produced natural and farm honeys and their comparison with the commercially available therapeutic honeys (including Medihoney® and manuka honey). **Methods** An agar dilution method was used to assess the activity of honeys against 20 bacteria and one yeast. The honeys were tested at ten concentrations ranging from 0.5% to 25%. **Results** All 20 bacteria were inhibited by all honeys used in this study with only the yeast *Candida albicans* not inhibited by the honeys at 20%. Little antibacterial activity was seen at honey concentrations <2%, with minimal inhibition at 5%. No honey was able to produce complete inhibition of bacterial growth at concentration up to 20% but with the increase in concentration few produces remarkable inhibition. Although Medihoney® and manuka had the overall good activity but the activity of naturally produced local honey was even better than those. The locally produced honeys by beekeepers had poor inhibitory activity for some, but not for all bacteria. **Conclusions** Honeys other than those commercially available as antibacterial honeys can have equivalent antibacterial activity if they produced through hygienic methods. The newly identified antibacterial honeys may prove to be a valuable source of future therapeutic honeys.

**Key Words:** Antibacterial, Natural Honey, Agar dilution, Medihoney.
Developing a SNP detection method, PS-TaqMan PCR, to detect CYP2C9*3 in south Chinese

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Keywords: PS-TaqMan PCR, SNP, CYP2C9

Objectives: To develop an appropriate tool for SNP detection in clinical laboratory, the previous TaqMan mismatch amplification mutation assay (TaqMA-MA) was modified to the primer special-TaqMan polymerase chain reaction (PS-TaqMan PCR) in order to satisfy the high-through and reliable characters. Moreover the developed tool was validated by detecting CYP2C9*3 polymorphism in south Chinese. Methods: We set up the double control standards to assure the PS-TaqMan PCR accurately, and investigated two cut-offs, homozygote cut-off and heterozygote cut-off, to distinguish genotype clearly. Moreover, the components of reaction mixture and condition of reaction were adjusted according to specificity. Results: The assay could exactly genotype the polymorphic template among the concentration gradient from 1 × 10^4 to 1 × 10^6 copy/PCR. The total 404 genomic DNAs of south Chinese were genotyped, 24 heterozygotes and 380 wild-type homozygotes were determined respectively and confirmed by adverse-directions sequencing. Conclusion: PS-TaqMan PCR was developed successfully and applied for CYP2C9*3 detection. The reliable, high-through and efficient tool could satisfy the clinical laboratory.

Preparation of Mycobacterium heparin-binding haemagglutinin and its application

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Objectives: M. tuberculosis produces on its surface modified protein antigens such as Mycobacterium heparin-binding haemagglutinin (HBHA). HBHA is a principal mycobacterial adhesin for epithelial cells, and is critical for escaping from the lungs and thus for extrapolmonary dissemination. At present, the study of mycobacterial adhesins are focused on the relationship between adhesins and host cells. It is only the beginning about application of mycobacterial adhesin in the diagnosis, treatment and prevention of TB. It is significant necessity that explore the function of mycobacterial adhesin HBHA in immune response of TB and its application in the diagnosis, treatment and prevention of TB. Methods: 1 HBHA was purified from mycobacterium bovis BCG by heparin-Sepharose chromatography, and bhA gene cloned into E. coli for expression of HBHA respectively. HBHA was injected into mouse and observe production of anti-HBHA antibody and its immunogenicity. 2 To select pulmonary and extrapolmonary tuberculosis patients, PPD(+) and PPD(-) healthy control, and detect serum anti-HBHA antibody levels by ELISA in different groups. 3 To explore whether HBHA can play a role in immunological prevention and treatment in animal tuberculosis Using mice as model. Results and Conclusion: 1 The bhA gene of M. tuberculosis have been cloned into PET-32a(+) expression vector, and got a high expression in E. coli. The recombinant HBHA was accumulated as inclusion bodies mainly in E. coli. The inclusion bodies were purified and acquired abundant HBHA protein. 2 Native HBHA protein were acquired from culture filters of M. tuberculosis. 3 The recombinant HBHA can increase antibody titer 100,000 times of immunized mouse compared with control group. It indicates that HBHA have good immunogenicity during purification process. 4 Both recombinant and native HBHA can be used as immunological diagnosis in TB and extrapolmonary TB. Native HBHA can increase specificity and sensitivity in TB diagnosis. 5 HBHA can play a role in immunological prevention and treatment in the mouse challenge model of M. tuberculosis.
between pulmonary tuberculosis patients and extrapulmonary tuberculosis patients had no difference. By using the nHBHA the different individuals were diagnosed and the results showed great power for nHBHA in accessory diagnosis.

50B

Evaluation of Two Chromogenic Media and Real-time PCR for Nasal Surveillance of MRSA

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Background: The prevalence of MRSA is epidemic in U.S. healthcare. An optimal methodology for accurate and rapid detection of MRSA is critical for surveillance programs as part of infection control. Most published reports vary and confound the determination of what method is most effective. This study is a comprehensive evaluation of 2 chromogenic media, with and without broth enrichment, and PCR to assess sensitivity (SN), specificity (SP), labor, and turn-around time (TAT).

Methods: A total of 500 nasal swabs collected for MRSA surveillance were selected based on PCR results (50% positive) for this evaluation. CHROMagar MRSA (CM), [BBL, BD], MRSASelect (MS) [Bio-Rad], and real-time PCR were used for MRSA detection. One swab was placed into a chromopeptidase (ACP) lysis solution and the other swab was used for culture to inoculate both plate media and then tryptic soy broth (TSB) for enrichment. TSB was incubated for 24h then plated to both media. Following the manufacturers’ procedure for interpretation, results of culture were determined at 24h and 48h for CM and at 24h for MS. PCR on the ACP lysate was done using the BD GeneOhm MRSA assay master mix (BD Diagnostics) and SmartCycler instrument. Blinded operators performed all testing.

Results: A total of 495 samples were analyzed, 5 were excluded due to possible culture contamination. A true positive result was defined as a sample with MRSA isolated from any culture or a PCR positive with a patient history of MRSA within the last year. Compared to the total of 186 true positives, the SN/SP for direct plating to CM at 24h was 78.5%/99.4%, at 48h was 81.2%/100%, and to MS was 78.5%/97.4%; for broth-enriched CM at 24h was 86.0%/99.7%, at 48h was 87.1%/99.7%, and MS was 90.3%/91.6%. The SN/SP for PCR based on our previous evaluations was 98.2%/97.5%. The labor involved for culture was documented for 100 specimens then averaged for a per sample value. For direct plating, the labor calculation at 24h for CM and MS were 30 sec per sample and CM at 48h added an additional 30 sec. While the labor time for PCR was just over 1.5 min per sample. Conclusion: The TAT for results for PCR can be as quick as 2h but depends on how frequently the test is performed. All culture methods have a minimum TAT of 18-24h. The labor effort was comparable for PCR and CM culture. Addition of broth enrichment increased the labor and TAT. Direct plating and broth enrichment to CM and MS have inferior SN compared to PCR (78.5-90.3% vs 98.2%; p<0.01). The SP of MS after enrichment was the lowest of the methods. The performance of laboratory tests must be considered when developing a MRSA surveillance program and real-time PCR offers a very sensitive, rapid and effective method.

51C

An Integrated Approach for Detection of Emerging Multiple-Drug Resistance in Glucose-Non-fermenting Gram Negative Organisms

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Background. Antimicrobial resistance in glucose-non-fermenting Gram negative organisms (NFs) is rapidly evolving, spreading globally and is increasingly difficult to accurately detect and characterize with phenotypic methods currently in use. Multiple-drug resistant organisms (MDRO) have become a worldwide issue with increased severity of infections, limited treatment options and infection control challenges. This study investigated an integrated approach using rapid antibiotic susceptibility results and advanced expert rules for the detection of MDRO in various NFs. Materials and Methods. Expert rules were derived based on published literature regarding definition of MDRO (resistant to at least 3 classes of antibiotics) for selected NF organisms. Rules with messages were implemented as specified in the literature. A total of 142 challenge strains were selected from the BD culture collection, including 92 strains of Pseudomonas, 45 strains of Acinetobacter, 2 strains of Stenotrophomonas, and 3 strains of Burkholderia. All isolates were tested in the Phoenix Microbiology System (U.S. Food and Drug Administration approved device for susceptibility testing) connected to EpiCenter System (BD Diagnostic Systems, Sparks, MD, USA) using NMIC/ID 101, 104 or 116 panel formats containing beta-lactams, beta-lactam/beta-lactamase-inhibitor combinations, aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole in various concentrations. The BDXpert system was enabled in the test system with CLSI M100-S17 (2007) interpretative criteria applied. Expertised laboratory reports were reviewed by at least two human experts for the accuracy of MDRO detection and messages. Reference microbroth dilution method was used to confirm the detected MDRO strains per CLSI recommended guidelines. The MDRO rate as alerted by the integrated system for each organism group was then tabulated.

Results. Of the 142 isolates tested, a total of 17 Pseudomonas aeruginosa strains (17/92; 18.5%) were flagged as MDRO by the integrated system. In addition, 1 Burkholderia and 1 Acinetobacter baumannii were detected as MDRO by the system. An Expert message was triggered that recommended consultation with an infectious disease practitioner for these isolates. The 2 MDRO Burkholderia and Acinetobacter isolates were also appropriately flagged as pan-drug resistant; the integrated system flagged an additional expert rule requiring user intervention and attention with these isolates. Conclusions. The integrated approach of Phoenix, EpiCenter and BDXpert systems reliably detects and reports MDRO in the NF isolates tested. Special messages, including therapy warnings, can be used to alert and communicate timely information for use by clinicians to achieve proper antimicrobial therapy and infection control for MDRO isolates.
Emerging Technologies of Medical Importance

52D

Shandong Cluster of Mycobacterium Tuberculosis Associated with Multi-drug Resistance and Increased Transmissibility in Rural Chinese Populations

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Emergence of drug-resistant tuberculosis (TB) has become a threatening concern for global health, and occurs predominantly in resource-limited countries. A population-based epidemiology study was conducted between April 2004 and June 2005 in two rural counties of Jiangsu Province, China. 351 Mycobacterium tuberculosis (MTB) isolates were collected for drug susceptibility testing and molecular characterization by MIRU, spoligotyping, IS6110 RFLP, and gene sequencing. 223 isolates (63.5%) were resistant to at least one anti-TB drug, including 53 (15.1%) multi-drug resistant (MDR) isolates. Spoligotyping found 243 isolates (69.2%) with Beijing family genotype. A major subgroup of the Beijing family, Shandong cluster identified by MIRU genotype 223325173535, accounted for 15.6% of Beijing family isolates. Cluster analysis with MIRU plus IS6110 RFLP genotyping found 62 (17.7%) isolates in 27 unique clusters. MDR-TB isolates were more likely to be clustered compared with pan-susceptible isolates (47.2% vs. 14.1%; odds ratio [OR], 4.72; 95% confidence interval [CI], 2.08 - 10.72), and overrepresented in the Beijing family compared with non-Beijing family isolates (18.5% vs. 7.4%; OR, 3.02; 95% CI, 1.35 - 6.73). Compared with other Beijing family isolates, Shandong cluster isolates showed significantly higher frequencies of multi-drug resistance (44.7% vs. 15.1%; OR, 6.18; 95% CI, 2.68 - 14.23), katG and rpoB mutations (26.3% vs. 5.4%; OR, 7.30; 95% CI, 2.60 - 21.51), and clustering (60.5% vs. 21.0%; OR, 6.14; 95% CI, 2.82 - 13.37). Our data strongly suggests that Shandong cluster MTB has increased transmissibility and genetic susceptibility of drug resistance, and may contribute to the high prevalence of MDR-TB in rural Chinese population.

53A

Trans-species assay for antibody to hepatitis E virus (HEV) for facile identification of HEV animal reservoirs

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How broadly enzootic is HEV, the causative agent of hepatitis E in humans? While antibody to HEV has been detected in a wide diversity of animals, the enzyme-linked immunosorbence assays used for such detection are almost always of an indirect format whose specificity is questionable. We have developed a double-antigen sandwich assay that obviates the requirement for the species-specific, 2nd-layer antibody that is obligatory to indirect assays. The antigen used for antibody capture, bound to the solid phase, is histidine-tagged p166s, a mixture of recombinant proteins derived from all four HEV genotypes, and the detector antigen is glutathione-S-transferase-tagged p166s, conjugated to horseradish peroxidase. Using pedigreed human samples, this assay achieved a sensitivity of 90% and specificity of 97% when compared with a commercially available anti-HEV assay. It was then applied to detect anti-HEV antibody in 2673 sera of wild animals captured in Georgia, Texas, Illinois, North Carolina and South Carolina, USA. Thirteen of 304 (4%) samples from swine and 112 of 501 (22%) samples from rodents were found to be anti-HEV-positive. None of the samples from deer (n=788), raccoons (n=311), bats (n=14), dogs (n=61), coyotes (n=300), skunks (n=92), horses (n=20), opossums (n=21), squirrels (n=63) and rabbits (171) were positive. Reverse-transcriptase PCR using a set of universal HEV PCR primers derived from open reading frame 2 of the HEV genome was applied to the 13 anti-HEV-positive swine samples, and 2 (15%) of them were found to carry HEV RNA. The sequences of the amplified products were identical and shared 91.6% and 90.5% identity to the human HEV strains US1 and US2, respectively, and 97.2% identity to the prototype US domestic swine HEV strain. All these sequences, which group with genotype 3 HEV sequences, are also clustered closely together, indicating recent evolutionary origin. The double-antigen sandwich trans-species assay should allow animal reservoirs of HEV to be identified more readily, thereby allowing the extent of cross-species transmission to be determined more precisely.

54B

The Detection and Characterization of Broad Groups of Respiratory Viruses, Including Pandemic Threats, by Mass Spectrometry Using the Ibis T5000 System


Background: The Ibis T5000 is an emerging infectious disease diagnostic technology, originally developed for biodefense, that couples broad-range PCR with electrospray ionization mass spectrometry (PCR/ESI-MS). It has been shown to be a very powerful method to detect and identify a broad range of bacteria. A broad variety of diverse viruses are known to cause respiratory disease in humans. We hypothesized that the Ibis T5000 strategy could be used to detect and identify the vast majority of viruses that cause respiratory disease in humans in a single assay. Methods: A set of broad range primer pairs was designed and organized into a single PCR/ESI-MS assay that encompass all known influenza viruses (Flu), including Flu A (all mammalian and avian types), Flu B and Flu C, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), adenoviruses, coronaviruses, bocaviruses and parainfluenzaviruses (PIV) types 1,3 and 2,4. The assay was tested with multiple isolates of each of the target viral families as well as clinical specimens from patients with respiratory symptoms. Results: The single assay described here was able to detect and specifically identify a broad variety of viruses from the each of the families described above and clinical specimens derived from patients with respiratory symptoms (Flu A, B, PIV 1-3, RSV and Adenovirus). Throughput was approximately 100 samples/24h. Limiting dilution experiments showed that the assay is sensitive to less than 100 viral genomes per reaction.
Results from a broader set of laboratory and clinical samples will be presented. Conclusions: Broad-range primers designed to groups of viruses coupled with mass spectrometry and signal processing software can be successfully used to identify a large number of diverse viruses that cause respiratory disease in humans.

55C

Evaluation of BD Phoenix Automated Microbiology System for Identification and Antimicrobial Susceptibility Testing of Clinically Important Bacteria

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Background. For optimal management of severe bacterial infectious diseases, such as septicemia, meningitis or endocarditis, proper treatment and infection control processes are critical. Timely and proper reporting of antibiotic susceptibility testing (AST) results is one of the key factors of providing accurate treatment and infection control. The BD Phoenix Microbiology System (BD Diagnostic Systems, Sparks, MD, USA) was evaluated for its accuracy and effectiveness of identification (ID) and AST of clinically important bacteria. Materials and Methods. A total of 171 fresh clinical isolates including 130 strains of Gram-Negative bacilli and 41 strains of Gram-Positive cocci (GPC) isolates were collected from clinical samples during one work week. Theses isolates included Enterobacteriaceae (90), Nonfermentative Gram-Negative Rods (NF-GN; 40), Staphylococcus (26), Enterococcus (4) and Streptococcus (11). In addition, a total of 129 stock strains from internal culture collection were evaluated, including 9 species of Streptococcus (26), 4 species of Enterococcus (36) and methicillin-resistant Staphylococcus aureus (MRSA; 34). Phoenix ID and AST testing were conducted according to the manufacturer’s recommended procedures. Traditional biochemical methods, the API ID system (bioMerieux), and the disc diffusion method (BBL Sensi-Disc) in accordance with the CLSI guidelines (M2-A9) were used as the reference methods. Results. The overall identification accuracy of Phoenix for the fresh isolates from clinical samples was 98.3% at Genus-level and 99.2% at Species-level in accordance with the reference methods, respectively. One strain of Acinetobacter baumannii was mis-identified as Burkholderia cepacia. The identification accuracy of Phoenix for the stock strains was 84.85% (28/33) for NF-GN, 97% (33/34) for MRSA, 100% (36/36) for Enterococcus spp and 92.3% (24/26) for Streptococcus spp. The categorical accuracies of Enterobacteriaceae and NF-GN were 96.9% and 88.2%, respectively, with minor error of 2.21% and 10.8%, major error and very major error of both less than 1%. For GPC, the overall categorical agreement was 99.5%. The time to results (TTR) of Phoenix ID was 3-6 hours. The TTR for Phoenix AST for Enterobacteriaceae and NF-GN were 7.5 and 10.7 hours respectively, whereas the TTR for GPC was 7.8 hours. Conclusions. Based on the evaluation of this study, the Phoenix system can provide early bacterial ID and accurate AST results used clinical microbiology laboratory. In addition, implementation of an automated system would improve the quality efficiency of a routine microbiology laboratory and provide alternative solutions for more difficult and labor intensive traditional bacterial ID and AST methods.

56D

Applications of Microfluidic Technology in Molecular Characterization of Human Enteroviruses.

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Microfluidic lab-on-a-chip technology represents a revolution in laboratory experimentation, bringing the benefits of miniaturization, integration and automation to many research-based industries. Here, we introduce this cutting edge technology into molecular typing of human enteroviruses. At present, gel electrophoresis is the most common method in analysis of PCR products. Although cost-saving, there are several limitations such as retrievable information from gel image, precise sizing and quantification of PCR fragments. These can be overcome by using a chip-based nucleic acid separation system. It has several advantages over the traditional slab gel in terms of speed of analysis, low sample consumption and reproducibility between different runs. The applications of this technology significantly reduce the turnaround time in processing and data analysis of routine enterovirus typing. In our current study, thirty cerebrospinal fluid (CSF) specimens were collected from patients suspected with viral meningitis infection for detection and characterization of enterovirus strains by PCR. Due to the minute amount of RNA present in CSF, it is therefore essential to set up a fast and sensitive method that allows quality control of extracted RNA. The RNA 6000 Labchip kits from Agilent thus enable us to estimate the quantity of total RNA and identify the degradation of mRNA which is crucial for subsequent experimental reactions. Quantified RNA was subjected to nested reverse-transcription PCR (RT-PCR) amplifications and sequencing reactions targeting enteroviral VP2 capsid protein's region. The performance of the chip-based system was evaluated by comparing the data obtained from Agilent 2100 Bioanalyzer to agarose gel analysis of the same PCR products. Results from the DNA chip revealed the presence of impurities in PCR amplifications which allows further optimization of PCR reactions. In addition to its high sensitivity, the precise size and concentrations of each PCR fragment can be measured with a sizing accuracy of more than 95%. The overall findings of this study suggest that chip-based analysis of PCR products may offer a reliable and significantly faster alternative to gel-based method.

57A

The cell surface expression of group 2 capsular polysaccharides in Escherichia coli: A function and computer-aided analysis of KfiB protein

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Capsular polysaccharide (CPS) has long been recognized as a significant virulence determinant in isolates capable of causing infection in humans and animals. In addition to studying how bacterial capsules are made and the roles they play, there are...
a number of important applications of this research. First, in pathogenic bacteria the synthesis of CPS represents an un-tapped target for the design of new anti-microbials. Secondly, CPS represent a repository of diverse polysaccharide molecules that can be potentially exploited to engineer new and interest-ing polysaccharide structures for use in a number of biomed-i-cal and food uses. Using the K5 antigen as a model system, our laboratory has shown that the biosynthesis of the K5 antigen involves 4 essential proteins (KfiA-D). The role of the three proteins (A,C,D) has been well defined. Our study established that the KfiB could be the initiating transferase, responsible for the transfer of N-acetyl-glucosamine (GlcNAc) to a membrane bound lipid which then allows the further addition of sugars by KfiA and KfiC. To investigate whether the detected incorporation of GlcNAc is associated with lipid, the lipid-associated material was extracted with 1-butanol. The fact that the radiolabelled product was 1-butanol-extractable, suggests KfiB is capable of transferring GlcNAc to a lipid. It has been demonstrated that the group 2 CPS are linked to phospha-tic acid (PA) at their reducing end. To investigate if KfiB was adding GlcNAc to PA, membranes were treated with phospho-lipase C (PLC) prior to extraction. PLC digestion had no detectable effect on incorporation of GlcNAc into membrane lipids. The effect of tunicamycin on transferase activity suggests that the lipid maybe undecaprenyl-phosphate. To support the structure-function studies of KfiB, the computer programme BLAST was used to find localised areas of similarity to other proteins. KfiB is a homologue of HyaE (P. multocida). The ability of the HyaE protein to complement the kfb mutation using the generated plasmid (pMP4) was assayed. Plasmid pMP4 was unable to complement the KfiB mutation and restore K5 capsule biosynthesis. To determine whether KfiB and its homologue HyaE contain a coiled coil, their predicted amino acid sequences were analysed by the COILS program. By compar-ing the MTK and MTDIK outputs, it can be seen that high probabilities for coiled coil formation (>90%) were seen with both matrices for KfiB (residues 360-445) and for HyaE (310-390 and 410-507). In summary, this study has established that the KfiB is an integral component of the membrane-bound K5 polybiosynthetic complex. Computer-aided analysis confirmed the notion that KfiB may well interact via coiled-coil interac-tions with other proteins of that complex. Also recognition of the KfiB as initiating transferase and its ability to transfer GlcNAc to lipid material is a significant step in our understand-ing of K5 biosynthesis.

58B

Neonatal Fecal Microflora in a Clinical Birth Cohort Study on the Development of Persistent Wheeze and Eczema in Early Life

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Background: The frequency of eczema and symptoms related with asthma are higher in North Europe than that in developing countries and eastern European countries. Hygiene hypothesis postulates that a changing early-life microbial exposure may increase the risk of allergic disease. The intestinal microflora is suggested to be a source for induction of immune deviation in infancy.

Objective: This study aimed to determine any discriminating differences in the intestinal microflora at 1 and 12 month of age between infants that developed persistent wheeze or eczema during the first 3 years of life in a prospective, longitudinal clinical study of a birth cohort.

Methods: A prospective birth cohort of 411 children born of mothers with asthma was studied in Copenhagen, Denmark. Symptoms of persistent wheeze and eczema were monitored during the first 3 years of life. Composition of fecal microbiota was evaluated by Polymerase chain reaction combined with denaturing gradient gel electrophoresis (DGGE). Band matching on DGGE profiles and cluster analysis were performed by BioNumerics software. The Principal Component Analysis (PCA) models were used to analyze the associations between band profiles and the development of persistent wheeze and eczema.

Results: At three years age, 348 subjects finished the follow-up and were included into the present analyses. 63 (20.93%) of 301, 151 (50.17%) of 301 children were diagnosed as persistent wheeze and eczema, respectively. Microflora was significantly more diverse at 12 month compared that with 1month (Band richness median 8 bands, range 2 to 20 vs. 6 bands, range 1 to 14; P<.005). In 1 month and 12 month samples, there was no significant difference of band richness between those infants who developed persistent wheeze, eczema, persistent wheeze or eczema and those who did not. Means 1 was 20.69% for 1month samples and 22.83% for 12month. No difference was showed between persistent wheeze or eczema cases and undiagnosed children at 1 and 12 month age. Similarity analyses of DGGE profiles did not reveal clustering of persistent wheeze, eczema, persistent wheeze or eczema cases neither at 1month nor at 12month. In 1 month and 12 month group, the curves of prevalence of dominant bands on DGGE profiles associated with persistent wheeze, eczema, persistent wheeze or eczema samples were similar with non-diagnosed samples. No sign of discrimination was found associated with the development of persistent wheeze, eczema, persistent wheeze or eczema group in 1month and 12month fecal samples with PCA.

Conclusion: Development of persistent wheeze and eczema is not preceded by a particular composition of the gut microbiota at 1month and 12month after birth.

59C

INNOVATIVE IMMUNO-DIAGNOSTIC TOOL FOR EARLY DIAGNOSIS OF ORTHOPAEDIC GRAFT INFECTIONS SUSTAINED BY STAPHYLOCOCCAL BIOFILMS

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BACKGROUND Staphylococci are a main cause in prosthesis infections. Early diagnosis -actually impossible due to paucity and unspecificity of symptoms- could allow resolution of these infections. We developed an immunoenzymatic assay (Lancet 2002) to detect serum IgM against staphylococcal slime polysaccharide antigens (SSPA) already tested in patients with
Staphylococcal Vascular Graft Infection (SVGI). Anti-SSPA IgG levels are unreliable; IgM levels increase during active biofilm infection sustained by Staphylococcci. This is a preliminary study on a possible use of the ELISA test on diagnosis and monitoring of Staphylococcal Orthopaedic Graft Infections (SOGI). METHODS SSPA is extracted and purified using a deposited patented strain by an original patented method. The test for IgM was performed on sera collected from 287 patients grouped as follows:
- 73 sera from patients with SOGI sustained by S. aureus and CNS
- 214 sera from controls (36 graft replacement in previous resolved SOGI, 14 non staphylococcal SOGI, 148 healthy controls and 16 patients with uninfected graft)

A cut-off value of 0.4 EU was established to distinguish between positive and negative results. Each serum sample was tested in triplicate in two different assays; value are expressed as means.

RESULTS IgG levels were higher than cut-off in all tested patients including controls. On the contrary nearly 67/73 patients with SOGI showed positive anti-SSPA IgM titres. Antibody titres higher than 0.4 ELISA units (EU) detected 91% of staphylococcal SOGI (estimated sensitivity) with an estimated specificity of 100% with 8.2% false-negative results and 0% false-positive results. The false negative group includes patients in which microbiological analysis were repeatedly positive with clear clinical signs of infections. The Positive Predictive Value is 100%; Negative Predictive Value is 97%.

DISCUSSION Anti-SSPA IgM represent a sensitive, specific, user-friendly, cheap and non-invasive diagnostic tool for SOGI caused by S. aureus and CNS. It represents the first available diagnostic tool for early diagnosis and for the detection of infection relapses after infected graft replacement (primary and secondary prevention of SOGI). The results obtained with 0.4 cut-off value would slightly modify towards a higher sensitivity and lower specificity in case we adopt a lower cut-off; it will depend on the choices of clinicians which option they will prefer.

60D

NON-INVASIVE EARLY DIAGNOSIS OF VASCULAR GRAFT INFECTION SUSTAINED BY STAPHYLOCOCCI: AN INNOVATIVE ELISA TEST

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BACKGROUND Patients harbouring permanent artificial medical devices (vascular grafts, heart valves etc.) have a major risk of bacterial colonization in the form of biofilm. Absence of symptoms is the hallmark of these infections subdolously evolving to levels incompatible with successful surgical replacement and medical eradication; early diagnosis could allow opportunite intervention and resolution of infection. The majority of these infections (70-80%) is generally caused by Staphylococcus spp that produce a slime polysaccharide. We developed an immunoenzymatic assay (IEA) to detect serum antibodies against Staphylococcal Slime Polysaccharide Antigens (SSPA) and tested its efficacy in patients with Staphylococcal Vascular Graft Infection (SVGI).

METHODS SSPA was extracted and purified from a deposited patent strain by an original method. The IEA test was performed on 187 sera previously collected from patients grouped as follows:
- 58 sera from patients with SVGI sustained by Staphylococcus spp
- 129 sera from controls (36 graft replacement in previous resolved SVGI, 12 non staphylococcal VGI, 43 healthy controls and 41 patients with uninfected graft)

We selected a cut-off value of 0.4 EU to distinguish between positive and negative results. Each serum sample was tested in triplicate in two different assays; value are expressed as means.

RESULTS Patients with ongoing staphylococcal SVGI showed higher anti-SSPA IgM titres than patients of other groups. Antibody titres higher than 0.40 ELISA units (EU) detected 98% of staphylococcal SOGI (sensitivity) with a specificity of 98%; the Positive Predictive Value (PPV) is 96%; Negative Predictive Value (NPV) is 99%. With this cut-off we found 2 false positives (1.55%) and 1 false negative (1.72%) Antibody titres higher than 0.35 (EU) detected 100% of staphylococcal SVGIs with an increase of false-positive results (diminished specificity).

DISCUSSION Our findings suggest that this methods represents an highly sensitive, specific, and non-invasive diagnostic test for staphylococcal SVGI. Since it is very easy to perform and cheap it can be considered as valuable diagnostic tool to be used in the routine clinical diagnosis and in the primary and secondary prevention of SVGI.

61A

Establishment and evaluation of a multiplex PCR assay for detection of pathogens causing major sexually transmitted diseases in Tianjin of China

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In China, laboratory diagnosis of sexually transmitted diseases (STDs) is still unsatisfying because assays for sufficient detection of the pathogens are not yet available in most of clinical laboratories. To meet the demand, Molecular Diagnostic of Vanguard (Vanguard Clinic, Tianjin, China) established a multiplex PCR assay (VG-M-PCR) to detect the five major STD pathogens (N. gonorrhoeae; NG; C. trachomatis; CT; U. urealyticum,UU; M. genitalium, MG; T. vaginalis, TV) simultaneously. The sensitivity, specificity, and reliability of VG-M-PCR were evaluated by comparing with those of other two multiplex PCR methods and obvious advantages were shown by VG-M-PCR. The assay of VG-M-PCR could reach to a sensitive level of 10 ng or 50 copies of pathogenic genome per reaction and amplify each of the five pathogens mixed with wide range of concentrations in a single sample. By combined a set of single PCRs that target the five pathogens at different genetic locations, VG-M-PCR assay was developed into a format of screening potential positive samples by a M-PCR and verifying detection results by a confirmation PCR (C-PCR), which has been accepted by clinical practitioners in several world-class institutes. The clinical practicability of VG-M-PCR was tested with a total of 155 specimens composed of 100 from STD clinic attendees and 55 from physical examinees. Of the 100 samples from STD attendees, 81 were pathogen-positive, among which 51 (63%) contained multiple agents; the positive detections were 53 for UU, 49 for NG, 31 for CT, 18 for MG, and 1 for TV. For comparison, the 100 STD samples were also
examined with culture methods: 46 were positive; 34 for UU, 22 for CT, MG for 4, NG for 1, and 14 (30%) were shown with more than one agents. Among the 55 specimens from examinees, 25 (45%) were positive and 4 (16%) were detected with multiple agents; the positive rates were 43.6% for UU, 3.6% for NG and CT, and 1.8% for TV. The results obtained from the two groups of clinical samples consisted with the logical expectation defined by the nature of the two populations and the data provided the field of STD pathogenesis with an epidemiological view in both STD patients and normal humans. The positive rates of PCR detection were much higher than those of conventional assays. In general, both laboratory evaluation and clinical test have demonstrated that VG-M-PCR possesses the sensitivity, specificity and reliability required for a competent M-PCR assay, therefore introducing this assay into clinical laboratories would definitely improve the laboratory diagnosis of STDs in China.

62B
Detection and genotyping of human papillomavirus in cervical specimens of Chinese women by a modified nested multiplex PCR

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Among the over 100 genotypes of human papillomaviruses (HPVs), a group of genotypes has been associated with development of cervical carcinoma; these are so-called high-risk HPVs (HRHPVs). In developed countries, the epidemiological understanding of HPV has been enriched with extensive studies on the viruses during last decade and the HPV genotyping by molecular techniques has been applied to identify women who are at increased risk of development or progression of a cervical lesion. Nevertheless, molecular assays for HPVs are not available in most of clinical laboratories in China and epidemiological characteristics of HRHPV in representative Chinese populations have not been obtained. We here describe our attempt to introduce a molecular assay of HPV’s into clinical application in Tianjin, China. A nested multiplex PCR (NMPCR) adopted from a literature was modified by optimizing the reaction system and conditions. The modification made the assay reaching to a sensitivity range from 103 to 106 copies of viral genome per reaction and readily detecting HRHPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 as well as HPV-6 or 11. Detection and genotyping of HPV were performed with clinical samples from three populations: P1, 110 women with abnormal diagnosis of cervical cytology, including Atypical Squamous Cells of Undetermined Significance (ASCUS), Low grade Squamous Intraepithelial Lesion (LSIL), High grade Squamous Intraepithelial Lesion (HSIL); P2, 104 female examinees normal in cervical cytology test; P3, 103 STD clinic attendees. The prevalence rates of HRHPV were detected by 56.4% in P1, 18.5% in P2, and 39.8% in P3; the HPV 6/11 was 10.9% in P1, 13.5% in P2, and 28.2% in P3. The samples with multi HRHPVs were 48.4% in P1, 3.0% in P2, and 27.4% in P3. The dominant genotypes in all the detected HRHPVs were HPV-16 (27.0%), HPV-56 (12.2%), and HPV-58 (10.4%). Grouping the specimens on diagnosis of cervical cytology, the positive rates of HRHPV were 90.9% (10/11) in HSILs, 64.0% (16/25) in LSILs, 52.2% (24/46) in ASCUSs and 18.3% (19/104) in normal examinees; the ratios of all HRHPVs/HPV-6 or 11 were 13:1 in HSILs and 4:1:1 in normal group. In conclusion, a sufficient molecular assay for HPVs was obtained by modification of a NMPCR and the method was demonstrated to be satisfactory through examining a number of clinical samples. Detection of HPVs in the samples revealed the distribution of HRHPV in the Chinese populations, identified the dominant genotypes of HRHPV causing infections, and confirmed the relationship between HPV infections and potential cervical lesions.

63C
Spatio-temporal progression of peritonitis and effects of treatment using in-vivo imaging

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Background: Peritonitis is a serious abdominal infection with high morbidity and mortality. Physical breech of the gastrointestinal tract and three dimensional spread of microorganisms inside the otherwise sterile peritoneal cavity causes peritonitis. Immune response to the disease leads to movement of the organ present in the peritoneal cavity in an effort to encapsulate the infection source and contain the infection. Spatio-temporal distribution of microbial load, and containment processes make the disease difficult to diagnose and treat using traditional lavage methods. Post treatment recurrence, due to the timing and inefficient spatial reach of the treatment, remains an important complication to peritonitis. Methods: In the present study, acute peritonitis was induced in 8 male Wistar rats by placing a fibrin clot containing 5 x 10⁸ cells of B. fragilis ATCC 25285 and bioluminescent E. coli Xen 14. After one or two days, the infected clot was removed and open abdominal lavage was performed. Spread of peritonitis was monitored using an in-vivo bioluminescent imaging up to 10 days after open abdominal lavage. Results: Bioluminescent in-vivo imaging showed that bacteria spread into the rat's abdominal cavity on day one after clot insertion, but on day two encapsulation of the clot confined bacterial spread. Quantification of the bioluminescent signal yielded the conclusion that the number of E. coli tripled on day one after clot insertion, while after two days a reduced bioluminescent intensity was measured. Treatment caused bioluminescent E. coli to spread over the peritoneal cavity, but within 11 days in-vivo imaging showed that the bacterial load had decreased 3-4 orders of magnitude. Delay in treatment by one day caused much larger abscesses. Conclusions: In-vivo imaging can be used to monitor the spatiotemporal behavior of peritonitis during three different stages of the disease process: initiation, treatment and follow-up. Moreover, in-vivo imaging allows to repeatedly image the same animal, therewith reducing variability and providing greater confidence in determining treatment efficacies using a small number of animals.
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Staphylococcal infection and colonization of pediatric intensive care unit patients: MRSA versus MRSE

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Until now the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in Estonia has been low (1-4%). In Sept 2006 - March 2007 a MRSA outbreak involving 10 neonates was registered in the PICU (pediatric intensive care unit) of Tartu University Clinics. In the case-control study we aimed to characterize MRSA isolates, to identify risk factors for MRSA acquisition and to compare those with the respective factors in MRSE colonization/infection. Material and methods. A total of 25 consecutive patients colonized with MRSA or MRSE were included. All of them were on admission younger than 72 hours and were empirically treated with penicillin and gentamicin for early onset neonatal sepsis. The following parameters were recorded: gestational age, birth weight (BW), mode of delivery, need for artificial ventilation (AV), duration of PICU stay and antibiotic treatment. Surveillance rectal and nasopharyngeal samples were collected on admission and twice a week until discharge from PICU and those from sterile sites when clinically indicated. All samples were cultured, incubated and identified on species level by API system or VITEK. Nuc gene was detected for S. aureus and methicillin resistance was confirmed by the detection of mecA gene by PCR. Clonal relatedness of the MRSA isolates was studied by PFGE and sequencing of the polymorphic region of protein A by spa typing. For assessing specific MRSA infection/colonisation risk factors, patients data of MRSA cases were compared with MRSE ones. Results. During the study period an equal number of patients were colonized with MRSA or MRSE (10 vs 15, respectively) and 3/10 and 4/15, respectively had bloodstream infections. In both groups the following characteristics: gestational age (<32 weeks vs 10/15, respectively), BW (<1500 g vs 8/15, respectively), vaginal birth (3/10 vs 7/15, respectively), number of twins (4/10 vs 1/15, respectively), main diagnoses, the duration of empiric antibiotic treatment (<72 h vs 1/15, respectively) and duration of PICU treatment (<7 d vs 4/15, respectively) were similar. MRSE colonization/infection cases were registered earlier than MRSA ones (during first week 14/15 of MRSE vs 5/10 of MRSA cases, OR=14; CI 1.2-150.9; p=0.023). The duration of AV in MRSA cases was significantly longer than in MRSE ones (7/10 of MRSA vs 3/15 of MRSE cases with AV duration more than 3 days; OR=9.33, CI: 1.46-56.479; p=0.034). All invasive and non-invasive MRSA strains but the first one had closely related PFGE type and belonged to the spa Type. Conclusions. Both MRSA and MRSE colonise mostly extremely premature babies and have with the exception of time of colonization and duration of AV have similar risk factors.

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Rapid Detection and Subtype Analysis of Avian Influenza in a Single-Tube Using LATE-PCR

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Pathogenic strains of avian influenza, particularly subtypes H5 and H7 continue to spread and evolve through out Eurasia and Africa, causing death and requiring culling of millions of birds. Several hundred people have died of avian influenza, primarily following direct contact with infected poultry, but there is reason to fear that relatively few genetic changes could lead to efficient human-to-human transmission, raising the specter of a devastating global pandemic. Rapid, accurate detection of the presence and type of influenza in people, birds, and other species is urgently needed, particularly in remote rural areas where people live in close proximity with their livestock. Smiths Detection, in collaboration with Brandeis University, is constructing a rapid, reverse transcription LATE-PCR assay for field detection and analysis of the low/high pathogenic variants of avian influenza subtypes H5 and H7, together with their N subtypes. The assay also detects Newcastle Virus if avian influenza is not present. An internal DNA standard and an external RNA control are included to guard against false negatives. All possible outcomes of this assay are generated in a single-tube that contains seven pairs of primers. Depending on which virus is present, sets of one to three single-stranded DNA target sequences are amplified and detected based on their patterns of hybridization to ten fluorescent probes in four colors. Hybridization of these probes to their specific targets is determined at the end of the amplification reaction by dropping the temperature to 70, 50, and 35 degrees Celsius. Proof-of-concept results for this sophisticated assay have readily been achieved using synthetic DNA targets added over six orders of magnitude and down to 10 target molecules. In addition, simultaneous reverse transcription of three viral RNA sequences in the same reaction, followed by amplification and detection of the corresponding cDNAs has also been achieved. The low and high pathogenic variants of H5N1 are unambiguously distinguished. Tests on the low and high pathogenic variants of subtype H7 are underway. This multiplexed assay will allow authorities to monitor the spread and possible evolution of H5N1 avian influenza in a single assay. This assay can be used in laboratory instruments, but is also designed for the BioSeq® system, a portable PCR instrument with automated sample preparation and analysis. The portability of the system, along with the automated sample preparation, will allow authorities to process suspect samples at the point of collection, allowing for a more rapid response for containment by eliminating the delays associated with sending samples back to a laboratory for analysis.
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Molecular epidemiology of methicillin-resistant Staphylococcal aureus in children

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Methicillin-resistant Staphylococcal aureus (MRSA) is a growing public health concern that has been associated with pediatric fatalities. This study investigated the genotypes of staphylococcal cassette chromosomal mec (SCCmeC) and Panton-Valentine Leukocidin (PVL) in MRSA strains isolated from Shanghai children’s hospital by PCR. Methods: 30 strains of MRSA were isolated from various clinical specimens in 2006. The antimicrobial susceptibility was measured by the agar diffusion method. SCCmec typing used a novel multiplex PCR assay allowing for concomitant detection of the methicillin resistance (mecA gene) analysis to facilitate detection and classification of all currently described SCCmec typing types, SCCmec Ia, I, III, IIIa, IVa, b, c, d, and V. PVL genotypes of the isolates was also determined by PCR. Results: mecA gene was positive in all of strains. Five isolates contained SCCmec Ia, and nine isolates contained SCCmec Ia. One isolate had SCCmec V, and another isolate was SCCmec IVa. Fourteen MRSA strains were non-typeable. The isolates with SCCmec Ia and SCCmec III were multiple-resistant to antibiotics. SCCmec Va and SCCmec V were susceptible to all antibiotics except β-lactams. Twelve isolates were PVL positive. The genotypes and subgenotypes of staphylococcal cassette chromosomal mec of twelve isolates of MRSA of PVL positive were SCCmec Ia (9 isolates), SCCmec III (7 isolates), SCCmec IVa (1 isolate), and SCCmec V (1 isolate). Conclusion: SCCmec Ia and SCCmec III are the major genotypes in MRSA of our hospital, these isolates are multi-resistant to antibiotics. PVL were encountered in SCCmec Ia and SCCmec III. Multiplex PCR is rapid, robust and capable of identifying the genotypes and subgenotypes of SCCmec in MRSA.

Study on the ESBLs and plasmid-mediated AmpC enzymes in CLSI ESBL-screening test positive but ceftazidime-susceptible E. coli and Klebsiella spp

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[Abstract] Objective: To study the ESBLs and plasmid-mediated AmpC enzymes in CLSI ESBL-screening test-positive but ceftazidime-susceptible E. coli and Klebsiella spp. Methods: Antimicrobial susceptibility testing were performed by Kirby-Bauer (K-B) method. The genes encoding ESBLs and plasmid-mediated AmpC enzymes were detected by PCR. Transfer of ESBLs or plasmid-mediated AmpC resistance was studied by conjugation experiments. DNA fingerprints were analyzed and interpreted by ERIC-PCR or PFGE as recommended by PulseNet protocol. Results: Of 18 isolates from Huashan Hospital, 11 were E. coli, 6 were Klebsiella pneumoniae and 1 was Klebsiella oxytoca. Results of antimicrobial susceptibility testing indicated all of 18 isolates were ESBL-screening test positive but susceptible to ceftazidime. PCR results indicated that 9 of the 11 E. coli isolates produced CMY-2 AmpC enzyme.
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Rapid detection of rpoB gene mutation from Mycobacterium tuberculosis via high resolution melting (HRM) curve analysis

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Background: Drug-resistant tuberculosis is a threat to tuberculosis control programs and community health. Rifampin, the highly effective bactericidal action of this drug against M. tuberculosis, has made it a key component of therapy. Virtually all isolates resistant to rifampin and related rifamycins have a mutation that alters the sequence of a 27-amino-acid region of the beta subunit of ribonucleic acid (RNA) polymerase. It has been reported that about 90-95% of rifampicin-resistant M. tuberculosis strains have rpoB mutations that concentrate in this code region, which is called the rifampicin resistance determining region (RRDR). We describe here a method for rapid screening RRDR mutation of rpoB gene using high-resolution melting (HRM) analysis. Method: Drug susceptibility testing (DST) on rifampin were applied for all isolates. The conventional PCR amplified rpoB gene RRDR assay was modified and optimized for the Rotor-Gene 6000 instrument (Corbett Life Science). HRM analysis on the Corbett Rotor-Gene 6000 instrument was used to test 80 known rpoB RRDR sequences obtained from 80 diverse isolates from China. Criteria for calling pairs of melting curves “same” or “different” were developed empirically by converting the data to difference graph format with one curve defined as the control. HRM curve comparison between runs was done to determine the portability of the method. The assay performance was assessed by DNA sequence results. Results: Isolates were found 52 strains were resistant to rifampin with MIC 4.0 to 8.0 µg/ml of drug susceptibility testing. HRM analysis of 80 PCR products amplified rpoB gene RRDR showed 43 samples with mutations. Compared with DNA sequence, HRM analysis matched very well with most of mutation of rpoB gene RRDR except 6 samples with mutation of GAC516GTC, CAC526GAC and TCG531TGG. All isolates HRMA showed different with control were resistant to rifampin. Conclusion: This study demonstrated that single- and closed-tube single-step method of HRMA for screening mutation of rpoB gene is rapid and simple and useful.

70B

Hepatitis B Virus Immune Status Among Health Care Workers at a Tertiary Care Hospital In South India.

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Background: Occupational exposure to Hepatitis B Virus (HBV) infection, a well known risk among health care workers (HCW), can be reduced by immunization with HBV vaccine. As a policy HB vaccine is offered voluntarily to all HCWs at employment at our Institute - a tertiary care hospital. Despite every effort to achieve 100% vaccination, a proportion of HCWs globally never volunteer to get vaccinated or complete the entire schedule. Aim: The present study was planned to assess the vaccination practices, HBV prevalence and immune status among the HCWs in a mass vaccination camp organized to commemorate the HBV eradication Day on 28th July 2006.

Subjects & Methods: 756 HCWs (332 males & 424 females) included in the present study, were tested for HBsAg & anti Hbs antibodies. A detailed H/o previous vaccination status and the number of doses received was recorded. The nonvaccinated HCWs were initiated into the vaccination schedule with an indigenously developed vaccine (SHANVAC-B) in 0,1 & 6 schedule. Results: HBs Ag was detected in 3 / 756 (0.39%). While 315 / 756 (41.6%) received vaccination in the past, 420 / 756 (55.5%) never received vaccination at all and 21 / 756 (2%) were not sure of their vaccine status. Of the vaccinated subjects, only 115 / 315 (36.5%) received all the 3 doses. Protective anti HBs antibody titres (≥ 10 mIU/ml) were detected in 83% of HCWs irrespective of their vaccination status. Although 33% of non vaccinated subjects had protective anti HBs titres, higher titers were observed in HCWs who completed the 3 doses of vaccine. In the followup of the present vaccination camp, only 42% subjects volunteered and completed the vaccine schedule. Conclusions: Despite the potential high risk & availability of free vaccine against HBV, there was poor compliance among the HCWs in this voluntary approach to achieve a high level immunisation. It is recommended that HBV vaccination be made mandatory among all HCWs to achieve 100% vaccine coverage and thereby protection against this preventable infectious disease in developing countries. Acknowledgements: The authors are grateful to M/S Shanta Biotechnics Ltd., for providing the Shanvac B vaccine for the study.

71C

Comparative study of safety and efficacy of 3rd generation Hepatitis B Vaccine (Sci B vac) with 2nd generation vaccine in healthy adults

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BACKGROUND: The Sci B vac vaccine is a novel third generation recombinant Hepatitis B vaccine, developed using mammalian Chinese Hamster Ovarian cells (CHO). It contains all viral surface polypeptides namely pre-S1, pre-S2 and S antigens. 10µg of pre-S plus vaccine is found equivalent to 20µg of Engerix B (Recombinant HBV vaccine) with respect to seroconversion rates and geometric mean titers (GMT).

AIMS & OBJECTIVES: The study was undertaken to evaluate the safety and immunogenicity of the Sci B vac vaccine in comparison to Engerix B vaccine in healthy adults. STUDY DESIGN: In this multicentric randomized open label parallel comparative study, 44 healthy adults (18 to 45 years of age) seronegative for HBV markers with normal haemogram & LFT were included from our center. They were randomly assigned to either Engerix B (Group 1) or Sci B vac (Group 2) and received 20µgs of Engerix B or 10 µg of Sci B vac intramuscularly in the deltoid region. The schedule of vaccination was 0,30 & 180 days. All the subjects were observed for 30 min after vaccination for any anaphylactic reactions. They were all given diary cards to record and report any side effects. AntiHbs antibody titres were estimated one month after each dose.
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sample independent “t” test using “SAS 8.2” version was used for statistical analysis. RESULTS: The Sci B vac was found to be clinically safe, well tolerated with no anaphylactic reaction. The protective seroconversion after each dose was found to be 13.6%, 52.3% and 88.2% in group 1 and 50%, 85.5% and 100% in Group 2. The GMT after 1st, 2nd and 3rd dose in Group 1 was 39, 66 and 2125 mIU/ml respectively and 57, 145 and 4002 mIU/ml in Group 2 respectively. The high GMT in group 2 was statistically significant (p = 0.001) as compared to group 1. CONCLUSION: The 3rd generation (CHO derived) HBV vaccine is highly immunogenic inducing significantly high seroprotection and GMT compared to standard comparator in healthy adults. ACKNOWLEDGEMENT: We are grateful to Shreya Life Sciences, Mumbai for providing the vaccines & financial Assistance.

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CLINICAL FEATURES & MOLECULAR DIAGNOSIS OF CHIKUNGUNYA FEVER FROM SOUTH INDIA

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Background: Emergence or resurgence of severe mosquito borne arboviral hemorrhagic fevers like the dengue virus & Chikungunya are being frequently reported in the Indian subcontinent for the past few years. Since the outcomes of these infections vary depending on the infecting agent (dengue has a high mortality rate) they pose a diagnostic dilemma for the clinician as these infections have similar manifestations and are difficult to distinguish from each other. Hence there is a need for a definite diagnosis and identification of the viral agent to prognosticate the outcome. Molecular diagnosis is an important tool to identify such new vector borne viral illness. An epidemic of an unprecedented magnitude of Chikungunya fever had occurred in many parts of India in the early 2006.

Subjects & methods: 296 patients, diagnosed as suspected CHIKV infection at the Nizam’s Institute of Medical Sciences, Hyderabad, Andhra Pradesh, South India, are included in this prospective study.

Results: The clinical symptoms were characterized by a triad of fever, rash and severe rheumatic manifestations. The illness was self-limiting with no mortality recorded in our study. There was a significant clustering of cases (40%) with young, adults being the worst hit. A significant female preponderance was also observed among our patients. The viral RNA could be detected in the early phase (1-2 days) of fever when there was a high level of viremia. While RT PCR was positive in 144 / 296 (48.6%) plasma samples, the RT LAMP was more sensitive and identified 20 additional cases of CHIKV (164 / 296, 55.4%). The latter test proved to be rapid, cost effective and can be adopted by ill equipped labs and field surveys. CHIKV was isolated from 20 / 32 randomly selected RNA positive samples. CHIKV IgM antibodies were detected in 21.5% of the cases.

Conclusion: This is probably the first report from India where the CHIKV isolates were phylogenetically identified as a novel variant of the East Central South African genotype.

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Macrolide-Resistant Mycoplasma pneumoniae with 23S rRNA Gene Mutation Emerged in Pediatric Patients in Shanghai, China

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Background Mycoplasma pneumoniae is one of the major pathogens in community acquired respiratory tract infections (RTI). Macrolides are the most important drugs in the treatment of RTI infections, especially in children. Recently the macrolide-resistance in M. pneumoniae had been reported in Japan, China and France. The mechanism of macrolide resistance is associated with transition mutations identified on the 23S rRNA gene. We aimed to determine the in vitro activities of macrolides and other antimicrobials against M. pneumoniae isolated from Shanghai and to investigate the mechanism of resistance to macrolides. Materials and Methods M. pneumoniae was isolated from clinical specimens of pediatric patients with low RTI. Susceptibility testing was performed for macrolides and other agents using broth microdilution method with SP4 broth. PCR amplification and sequence analysis of 23S rRNA gene were performed for all M. pneumoniae strains. Results Twenty M. pneumoniae isolates were obtained from 585 samples since October 2005 to March 2006. Eleven (11/20, 55%) clinical isolates were resistant to three macrolides tested, erythromycin and clarithromycin and azithromycin, with MICs of equal or over 128 µg/ml. All of M. pneumoniae isolates were susceptible to the tetracyclines and fluoroquinolones tested. All tetracyclines had good activities against M. pneumoniae with similar MIC values. Moxifloxacin was more active than ciprofloxacin and levofloxacin. All of the macrolide-resistant M. pneumoniae strains harbored an A-to-G transition mutation at position 2063 in domain V of 23S rRNA gene, as had been reported previously. Conclusions The M. pneumoniae resistance rate to macrolides was high in Shanghai, China. The mechanism of resistance was related to transition mutation of 23S rRNA. The number of strains in this study is limited and further studies are needed to clarify the prevalence of macrolide-resistance in M. pneumoniae.

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Rapid Detection of Pathogenic Vibrio parahaemolyticus by a Sensitive and Specific Duplex PCR-Hybridization Probes Assay using LightCycler

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A real-time PCR-hybridization probe assay was developed for rapid and specific detection of thermostable direct haemolysin-producing V. parahaemolyticus (V. parahaemolyticus). Primers and hybridization probes were designed to target the toxR and tsh2 genes. Mismatches were introduced in the tsh2 primers
for specific amplification of the target. The 3’ ends of donor probes for both genes were labeled with fluorescein. The 5’ ends of recipient probes for tdh2 and toxR were labeled with LC Red 640 and LC Red 705, respectively. The real-time assay was evaluated against conventional biochemical tests and the KAP-RPLA kit (Denka Seiken Co, Japan). toxR and tdh2 were detected in 100% and 91% of clinical V. parahaemolyticus isolates (n=118), respectively. Specificity and sensitivity of the real-time assay for toxR and tdh2 were 100%. Dynamic range of detection for toxR was 107 to 1011 CFU/mL, and that for tdh2 was 107 to 106 CFU/mL. The LightCycler assay described is sensitive and highly specific for detection of pathogenic V. parahaemolyticus in a single reaction tube within 80 min. The assay developed allows accurate detection of pathogenic V. parahaemolyticus, which is valuable for rapid tracing of infection source during outbreaks.

75C

Comparative study of factors effecting pathogenicity of Hydro pericardium Virus a type of Fowl Adeno Virus group-I

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Hydro pericardium Syndrome primarily affects the broilers between the ages of 3-5 weeks. The present study has been planned to study the pathogenicity of F Adv-4 by inoculation of different age groups of broiler birds through different parenteral and oronasal routes. The liver homogenate suspension prepared from infected liver samples and cell culture propagated infectious agents were used to infect the susceptible broiler birds via parenteral routes and through oronasal routes. For this purpose two experiments were designed as Experiment I and II. In Experiment I 25-day old broiler birds were inoculated with different dilutions of liver homogenate and cell culture propagated HPS virus through intramuscular and oral routes. Similarly in Experiment II the one-day-old, l-week-old, 2-week-old, 3-week-old and 4-week old broiler chickens were inoculated with the original dilution (107) of same liver homogenate and cell culture propagated HPS virus through SIC and oral route. The birds were kept under observation for recording morbidity and mortality. In Experiment I the liver homogenate caused 64% mortality in broiler birds of the Group A through intramuscular route, while 33.33% mortality in broiler birds of Group B through oral route. The cell culture propagated HPS virus caused 60% and 13.33% mortality in broiler birds of Group C and D through intramuscular and oral routes, respectively. In Experiment II none of the day old chick died from Group A inoculated with liver homogenate and cell culture propagated HPS virus through SIC and oral route. The liver homogenate and cell culture propagated HPS virus caused high mortality in different age groups of broiler birds through SIC route than oral route. The blood samples were collected from the broiler birds before and after infection and various hematological parameters such as Hemoglobin and packed cell volumes were studied. The values were statistically analyzed and showed highly significant (P<0.05) reduction indicating anemia. The values of hemoglobin and packed cell volume of the broiler birds inoculated with infectious liver homogenate showed highly significant reduction than the birds inoculated with cell culture propagated HPS virus. The results indicated that the liver homogenate is more pathogenic than cell culture propagated HPS virus. This may be due to the adoptability of the original FAdVs after continued passages in the culture of chicken embryo liver cells.

Key Words: pathogenicity, homogenate suspension, cell culture propagated, HPS virus

76D

Multiplex Real-Time PCR for Rapid Detection and Identification of Toxic Vibrio cholerae

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Cholera is a potentially epidemic and life-threatening secretory diarrhea characterized by numerous, voluminous watery stools, often accompanied by vomiting, and resulting in hypovolemic shock and acidosis, while some members of Vibrio cholerae species also cause mild or apparent infections or isolated outbreaks of milder diarrhea. Thus, detection and differentiation of Vibrio cholerae species are critical for cholera prevention. To meet this requirement, we developed a 4-color multiplex real-time PCR assay targeting four markers of Vibrio cholerae using modified molecular beacons probes. hlyA gene was tested for the presence of Vibrio cholerae, rfb gene was for the presence of O1 group, wbbR gene for O139 group, and ztcA for cholera toxin production. To minimize primers interactions in the multiplex PCR, we used a so-called homo-tag assisted non-dimer strategy. The results showed that this multiplex real-time PCR could detect down to 2 copies genomic DNA per reaction for each gene with dynamic range up to 2×107 copies. The entire assay could be completed within 3 h and a simple heating lysis could be used for template preparation. The clinical sensitivity and specificity of this method were evaluated using 42 strains of Vibrio cholerae-related bacterium from various sources and proved to be both 100%. The result demonstrated that this assay could be used in rapid detection and identification of toxic V. cholerae from various resources.

77A

Real-Time PAP Detection of Rare Mutations in Isoniazid-Resistant Mycobacterium tuberculosis in Sputum Samples

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Isoniazid is one of the most effective first line anti-tuberculosis drugs and its resistance causative mutations have been well characterized. The predominant mutation conferring isoniazid resistance in Mycobacterium tuberculosis is katG315 (AGC→ACC). Obviously, early identification and quantification of isoniazid-resistant bacilli will be of great value in guiding and monitoring treatment process. However, it is technically difficult to detect a small portion of mutation-containing TB in the presence of large number of wild-type in clinical samples. Here we described a real-time pyrophosphorolysis activated polymer-
ization (PAP) method to address this problem. PAP has been proven to be extremely specific to rare mutation detection, however, it needs laborious post-amplification manipulations and could not give quantitative information. We upgraded PAGE-based PAP to real-time PAP, and achieved the detection limit down to 5 copies per reaction, and the selectivity up to 1:10000. Real-time PAP was validated with 105 TB-positive sputum samples, among which 100 samples gave concordant results with both probe-based real-time detection and sequencing, 3 samples were detected by real-time PAP as mixture of predominant wild-type and the mutants, but were mutants by real-time PCR and wild-types by sequencing, 1 sample was detected by real-time PAP as mixture of dominant mutant and wild-type but was detected as mutant by real-time PCR and as wild-type by sequencing, 1 sample was detected as wild-type by both real-time PAP and real-time PCR but exhibited an infrequent katG315 (AGC→AAC) mutant-type allele by sequencing. These results showed extra advantage of real-time PAP in detecting rare species compared with current methods. We concluded that real-time PAP will be a very useful tool for tracing and monitoring the development of drug-resistant mutants during anti-tuberculosis therapy.

78B
Diagnostic tools to study and detect helicobacter infections in laboratory mice

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Introduction. Mouse models for a great number of chronic infectious diseases, experimental vaccine and other therapy studies are widely used. Some bile-tolerant enterohepatic Helicobacter species have been associated with chronic hepatitis, colitis and IBD-like human disease in naturally and experimentally infected mice, emphasizing the necessity to use Helicobacter-free animals, since sub-clinical infections and inflammation may interfere with various disease study models. Immunoblot assays (IB) were developed to detect anti-Helicobacter antibodies and a PCR-denaturing gradient gel electrophoresis (DGGE) assay to detect DNA of enterohelobacter Helicobacter species.

Material and Methods. Serum antibody responses to cell surface proteins CSP’s of H. bilis, H. hepaticus and H. ganmani were analysed in 198 mouse sera obtained from five academic animal facilities in Southern Scandinavia. CSP’s were extracted by a 0.2 M acidic glycine buffer, pH 2.2, separated by gradient SDS-PAGE and electrophoretically transferred to PVDF membranes. Seven sera were from a group of mice experimentally infected with H. bilis. From 56 of the animals, lower bowel tissue specimens were available and analysed by PCR-DGGE. Results of the PCR-DGGE were compared with immunoblot results of corresponding sera.

Results. Specific antibody reactivity to H. bilis was detected in 15/196 (8%) animals, to H. hepaticus in 39/194 (20%) and to H. ganmani in 48/198 (24%) of the mice sera. These results were compared with PCR-DGGE analyses of corresponding tissue samples and agreement between the two diagnostic tests was found in 95% for H. bilis, in 86% for H. hepaticus and in 77% for H. ganmani. The PCR-DGGE also detected DNA of H. typhlonius, H. sp flecspirae and H. rodentium but antibody responses to these species were not evaluated in this study. Conclusions. Infection with enteric Helicobacter seems to be common in laboratory mouse colonies. The chronic inflammation induced by intestinal and hepatic Helicobacter infections may interfere with results of experimental studies of various diseases. Serological tests with high specificity and sensitivity, such as immunoblot, has a great potential as a diagnostic tool in health monitoring of laboratory mice colonies and discriminate transient PCR-positive gut colonization from infected animals.

79C
Controlling fungal pathogens by screening synergistic microbial metabolites

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The high mortality of fungal infections in immunocompromised patients and the limited availability of highly efficacious and safe agents demands the development of new antifungal therapeutics. However, infectious pathogens are composed of complex networking systems with redundant, convergent and divergent signaling pathways. To control fungal infection, multicomponent therapies along the disease pathway may need to be manipulated simultaneously for an effective treatment. Many drugs could be more effective at a reduced dosage if low dosages of other synergistic compounds are introduced simultaneously. In order to rapidly discover such agents, we developed a high throughput synergy screening (HTSS) strategy for novel microbial natural products. Specifically, a microbial natural product library was screened for hits that synergize the effect of a low dosage of ketoconazole (KTC) that alone shows little detectable fungicidal activity. Through screening of twenty thousands of microbial extracts, twelve hits were identified with broad spectrum antifungal activity. Seven of them showed little cytotoxicity against human hepatoma cells. Fractionation of the active extracts revealed beauvericin (BEA) as the most potent component as it dramatically synergized KTC activity against diverse fungal pathogens by a checkerboard assay. Significantly, in our established immunocompromised mouse model, combinations of BEA and KTC prolonged survival of the host infected with Candida parapsilosis and reduced fungal colony counts in animal organs including kidneys, lungs and brains. Such an effect was not achieved even with the high dose of 50 mg/kg KTC. HE (hematoxylin/eosin) and PAS (periodic acid-Schiff) staining of the recovered tissues revealed new mechanism of synergy compounds. These data support synergism between BEA and KTC and thereby a prospective strategy for antifungal therapy.

80D
Lateral Flow Immunoassay Using Novel Fluorescent Silica Nanoparticles for Fast, Ultrasensitive Quantification of Virus

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We report the application of a novel fluorescent silica nanopar-
81A
High Resolution Melting for Rapid Screening of Rifampin-Resistant Mutations in Mycobacterium Tuberculosis

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Rifampin resistance is regarded as surrogate of multidrug-resistance of tuberculosis. So far, a variety of detection methods for rifampin-resistant mutations have been developed. We reported here a significantly cost-effective method for screening mutations occurred in the 81-bp rpoB gene accounting for over 95% of the mutations associated with rifampin resistance. This high resolution melting (HRM) method needs only PCR in the presence of a saturating dye and the PCR product is directly analyzed on the PCR machine without opening the caps of the reaction tubes. We constructed 7 templates containing those commonly occurred mutations, i.e., codon 531 (TCG→TTG), codon 533 (CTG→CCG), codon 523 (GGG→GAG), codon 516 (GAC→GTC), codon 526 (CAC→GAC), codon 516 (GAC→TAC), and codon 511 (CTG→CCG), and used them to screen flanking primer pairs capable of giving distinctive HRM curves of these mutations from the wild-type. We chose one pair of primers that amplify a 189-bp region covering the 81-bp of rpoB that could detect all the 7 variants from the wild-type with 100% confidence. We observed that this system could identify mutant templates as low as 5% in the presence of wild-type templates and could amplify a single copy of all the templates. When evaluated with clinical samples, we observed that the quality rather than the quantity of the template DNA had substantial effect on the analysis result, and purified DNA could always give reproducible results. The evaluation work involving a large number of clinical samples is still being undertaken and the final results are still to come. Nevertheless, the results obtained so far are very encouraging and have made us believe that HRM would be a very competitive alternative for rifampin-resistance screening in clinical settings.

82B
Identification of Multiple Foodborne Pathogens by Multicolor Combinational Probe Coding Technology in a Single Real-Time PCR

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The availability of rapid, specific, and high throughput assays to detect the presence or absence, or the degree of contamination of pathogens, has become increasingly important for food safety and environmental protection. Real-time PCR assays have been widely used for detecting foodborne pathogens but have been much less frequently applied in species identification, mainly because of the low number of species they can distinguish in one reaction. We presented here a novel strategy termed multicolor combinational probe coding (MCPC) to increase the number of targets that can be distinguished in a single real-time PCR for rapid and reliable species identification. With MCPC, as many as 15 target sequences could be identified and discriminated in a single tube. We first demonstrated the feasibility of MCPC in the differentiation of 9 foodborne pathogens (E. coli & Shigella spp., Salmonella spp., Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Vibrio cholerae, V. para- haemolyticus, Proteus spp. and Streptococcus pyogenes ) combined with broad-range ribosomal RNA real-time PCR, in which 3 pairs of primer and 10 probes were included in a single tube. We further extend to differentiate 10 foodborne pathogens (S. aureus, L. monocytogenes, Salmonella Typhi, Shigella, E. coli O157:H7, enterotoxigenic V. cholerae, V. para- haemolyticus, V. pyogenes, enterotoxic and emetic B.cereus , and Y. enterolitica) using target-specific genes. In such a system, 10 pairs of targeted-specific primers, 10 target-specific probes, and 1 universal primer were included in one reaction. Finally, we validated MCPC for differentiation of 7 pathogenic vibrios based on their molecular polymorphisms (V. cholerae O1, V. cholerae O139, total and pathogenic V. parahaemolyti- cus, V. vulnificus, V. mirmicus, V. alginolyticus and V. fluvialis). Our results showed that all these three detection systems, the specificity and sensitivity were both 100% via blind assessment of bacterial strains including both clinical and environmental isolates. The detection limits for the later two assays ranged from 2 copies to 100 copies of genomic DNA per reaction. We concluded that MCPC would be accepted as a high throughput strategy suitable for rapid and reliable identification of foodborne pathogens in both clinical and environmental samples.

83C
Identification of Multiple Foodborne Pathogens by Species-specific Real-time PCR with High-resolution Melting Analysis

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Rapid and cost-effective methods for detecting foodborne pathogens have become increasingly important for food poison...
control and prevention. Real-time PCR assays have been widely used for detecting foodborne pathogens but have been much less frequently applied in multiple species identification, mainly because of the low number of species they can distinguish in one reaction and the high cost needed in their establishment. We presented here a cost-effective method that is capable of detecting multiple foodborne pathogens in a single real-time PCR. Our methods combined species-specific amplification and high-resolution melting analysis and could tell the existence of as many as 10 pathogens after real-time PCR. The 10 pathogens, i.e., S. aureus, L. monocytogenes, Salmonella Typhi, Shigella, E. coli O157:H7, enterotoxigenic V. cholera, pathogenic V. parahaemolyticus, S. pyogenes, enterotoxigenic and emetic B. cereus, and Y. enterocolitica, were amplified based on their species-specific gene sequences with distinguishable melting temperature (Tm) of their amplicons. Two separate 5plex real-time PCRs were conducted in the presence of SYTO13. Following PCR, HRM profiles of each sample were obtained automatically through the preset HRM procedure. This method could not only tell the identity of each pathogen but also could give co-infection information. The reliability of the results were improved with the inclusion of Tm controls in the reaction but usually these controls could be omitted if the templates DNA prepared are relatively pure. Totally 200 samples from a variety of sources were tested and compared with culture-based methods, and 100% agreement were achieved. This method is so far the most inexpensive real-time PCR methods for foodborne pathogens identification and has great potential for routine use.

84D

Real-Time PCR Detection of Rifampin-Resistant Mycobacterium Tuberculosis in Sputum Samples

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Current clinical assays for determining antibiotic susceptibility in Mycobacterium tuberculosis require several weeks to complete due to the slow growth of the bacilli. Here we reported a real-time PCR protocol suitable for detection of rifampin-resistant species in sputum samples that could be finished in less than 5 h. Our protocol consisted of two consecutive real-time PCR steps. First, IS6110 was detected from DNA template extracted from sputum samples to confirm the existence of M. tuberculosis. Second, for those IS6110-positive samples, the 81-bp core region of rpoB gene was screened by a 4-color real-time PCR using four overlapping displacing probes labeled with Cy5, HEX, ROX, and FAM, respectively. The mutation was identified by the decline of fluorescence from any of the 4 fluorophors. This protocol could detect down to 1 copy of M. tuberculosis per reaction and could identify all rifampin-resistant mutations in the detect sequence. We validated this protocol with 129 smear-positive sputum samples. The results showed that all the 129 samples were detected IS6110 positive, 106 of them were wild-type, and 23 samples were mutation-containing. These samples were again sequenced in their core region of rpoB gene and the results obtained from 123 samples gave the concordant results with real-time PCR except for 6 samples that failed to give sequencing results. This protocol could be easily adapted to clinical settings equipped with real-time PCR machines, and it is simple to conduct, cost-effective, and is both sensitive and specific in mutation detection. The established protocol is now being evaluated for application in clinical settings.

85A

PNA FISH for Rapid Identification of Pathogens Directly from Blood Culture Bottles

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PNA FISH Principle: The PNA FISH method enables rapid detection of microorganisms directly from positive blood culture (BC) bottles by fluorescence in situ hybridization (FISH) using peptide nucleic acid (PNA) probes targeting species-specific rRNA sequences. Slides are prepared directly from BC bottles by combining a drop of the culture, and a drop of a fixative solution. Fixed slides are processed through a 90 min hybridization step, followed by a 30 min wash. Slides are scored on a fluorescence microscope equipped with the appropriate filter. The entire procedure requires about 2.5 h, including roughly 20 min of hands-on time, to process 10 slides. All kits use the same reagents, except for the PNA probe solution. Gram-positive cocci in clusters (GPCPC) are detected as either green (S. aureus), or red (coagulase-negative staphylococci, CNS), by S. aureus/CNS PNA FISH. Gram-positive cocci in pairs and chains (GPCPC) are detected as either green (E. faecalis), or red (Non-faecalis enterococci), by E. faecalis/OE PNA FISH. Yeast are detected as either green (C. albicans), or red (C. glabrata), by C. albicans/C. glabrata PNA FISH.

PNA FISH Performance: In studies of combined 275 GPCPC-positive and 54 other positive routine BC from four labs, the S. aureus/CNS PNA FISH method had 100% (94/94) sensitivity for S. aureus, and 98.9% (179/181) sensitivity for CNS; the combined assay specificity was 96.9% (62/64). In studies of combined 161 yeast-positive routine BC from five labs, the C. albicans/C. glabrata PNA FISH method had 98.6% (68/69) sensitivity for C. albicans, and 100% (29/29) sensitivity for C. glabrata; the combined assay specificity was 100% (63/63). For 305 true and 48 spiked BC positive for GPCPC from five labs, the E. faecalis/OE PNA FISH method had 98.4% (125/127) sensitivity for E. faecalis, and 96.6% (56/58) sensitivity for other enterococci; the combined assay specificity was 99.29% (127/128). Impact on therapy: Since most instances of CNS in positive blood cultures are due to contamination of the culture with skin flora, this kit helps reduce the number of patients who unnecessarily receive antibiotic therapy. Since E. faecalis are usually sensitive to vancomycin, whereas, non-faecalis enterococci may be resistant, this kit helps to direct therapy prior to the availability of susceptibility data. C. albicans are usually sensitive to fluconazole, whereas, C. glabrata may be resistant; this kit helps to direct therapy prior to the availability of susceptibility data. Using these three PNA FISH kits, the majority of positive BC bottles can be identified to the species level. Use of PNA FISH kits for the detection of staphylococci, enterococci and yeast species may impact the use of antimicrobial agents. Conclusion: PNA FISH provides rapid and accurate methods for identification of positive BC bottles that provides important information for patient therapy and management.
86B

GENETIC DIFFERENCES IN VIRULENCE FACTORS USED AS DIAGNOSTIC MARKER TO DISTINGUISH ESCHERICHIA COLI O157:H7 LINEAGES

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Background: Escherichia coli O157:H7 is an important food- and waterborne bacterial pathogen associated with outbreaks of hemorrhagic colitis and the haemolytic uremic syndrome (HUS). Two lineages of E. coli O157:H7 have recently been identified which appear to differ in virulence based on the frequency with which they are associated with human disease - lineage I strains are more commonly associated with human disease than lineage II strains. Objectives: In this study, genetic determinants of two key virulence factors, Shiga toxins (Stxs) and the locus of enterocyte effacement (LEE) of lineage I and II E. coli O157:H7 strains were compared. Design: The upstream nucleotide sequences of the stx1 and stx2 genes were investigated. Additionally, the nucleotide sequence of the bacterial prophage insertion sites for both Stxs and the LEE were determined. Lastly, RFLP patterns of the tir gene in the LEE across the two lineages were compared. Results: 1) The upstream nucleotide sequence of the stx2 gene of most lineage II strains (93% of 41 strains) differed in the Q antiterminator protein genes from lineage I strains (97% of 62 strains). 2) RFLP and DNA sequencing showed that lineage I strains carry the stx2 while lineage II strains carry the stx2C variant gene. 3) The insertion site for the stx2-carrying bacterial prophage in lineage II strains is sbcB while that of lineage I strains is the wrbA gene. 4) The insertion site for the stx1-carrying prophage and the LEE are the yerV and sedC genes, respectively in both lineage I and II strains. 5) The LEE tir in most lineage II strains (93% of 46 strains) has two 17 bp repeat sequences whereas this sequence is not repeated in the most lineage I strains (97% of 62 strains). Conclusion: The genetic differences noted in these important virulence factors of E. coli O157:H7 may account for differences in host range and virulence of these two E. coli O157:H7 lineages and also serve as useful diagnostic markers.

87C

Global Laboratory-based Influenza Surveillance through the United States Department of Defense

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The Department of Defense (DoD) Global Emerging Infections Surveillance and Response System (DoD-GEIS) sponsors influenza and respiratory illness surveillance at a total of 275 sites in 56 countries. The global laboratory-based system centers on 2 DoD respiratory disease reference laboratories, the Air Force Institute for Operational Health (AFIOH) and the Navy Health Research Center (NHRC). The AFIOH uses a sentinel site methodology while NHRC conducts population-based febrile respiratory illness surveillance. Both of these DoD reference centers are Clinical Laboratory Improvement Program (CLIP) compliant. Influenza viral identification is initially accomplished through classic immunological fluorescent-based staining while subtyping of the hemagglutinin gene (HA) is carried out through specific RT-PCR reactions. Genotypic characterization is accomplished through cDNA sequencing and subsequent analysis of the influenza A HA gene and to a lesser extent the neuraminidase gene. Phylogenetic analysis of HA evolutionary relationships is conducted on these DNA sequences using DNASTAR software, Paup sequencing supplement, and NCBI’s influenza-specific annotation, alignment, and phylogeny tools. Virus isolate information, subtyping and notable molecular characteristics are reported to DoD-GEIS, DoD Health Affairs, collaborators, and sentinel sites. Viral isolates of interest are shared with the US CDC. The global nature of the GEIS influenza surveillance system allows for early detection of influenza A antigenic variants before they reach the US. During the 2006-2007 season, over 1,100 influenza A/B specimens were screened, subtyped and sequenced. In July 2004, GEIS surveillance efforts led to the identification of an influenza A H3N2 variant by AFIOH from an outbreak at a Nepal surveillance site. Based on collaborative studies between AFIOH and CDC, these antigenic variants were predicted to have reduced reactivity with antibodies elicited by the current US vaccine. AFIOH /CDC contributions led to changes in the H5N2 formulation of that year’s northern hemisphere trivalent vaccine. NHRC has similar laboratory capabilities although NHRC has the added capability to perform molecular DNA analysis of influenza and other infectious diseases using the Ibis Pharmaceutical T5000. With heightened awareness for a future pandemic influenza event, the DoD-GEIS surveillance network is one of the only systems prepared to detect novel viral isolates on a global scale.

88D

Direct PCR detection of viral pathogens in crude clinical samples using novel blood-resistant Taq mutants

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A major problem with PCR-based diagnostic tests of blood samples is the false-negative or low sensitivity reactions caused by PCR inhibitors. Hemoglobin and a serum IgG fraction known to inactivate Taq DNA polymerase, which can be inhibited in the presence of less than 0.1% whole blood. Therefore, various DNA purification methods and kits have been used to remove PCR inhibitors prior to PCR. However, residual amounts of inhibitors may still compromise the PCR detection. For example, 14% false-negatives for HBV have been reported in routine tests. Furthermore, these pre-treatment steps are generally costly, time-consuming, and increase the likelihood of sample cross-contaminations or DNA losses. As an alternative to these methods we attempted to generate blood-resistant mutants of Taq DNA polymerase which are able to overcome PCR inhibitors. Two mutant clones, obtained from a mutagenized library of the gene, remained functional in the presence of at least 20-25% blood or serum and eliminated the need for DNA purification before PCR. We found that as little as a single codon change in Taq can provide the useful
Emerging Technologies of Medical Importance

phenotype. Here we demonstrate the application of the novel Taq mutants for direct PCR detection of viral DNA from intact viruses in blood samples. Primers were designed to amplify a 650 bp DNA fragment of the duck hepatitis B virus P gene, a 140 bp fragment of HSV-2 UL30 gene, a 140 bp fragment of the adenovirus-5 Hex gene, and a 190 bp target of the EVM106 region of enteroviral virus (mousepox). Using the blood-resistant mutant enzymes in serial dilutions of the test viruses side by side with equivalent amounts of kit-purified viral DNA, we obtained equally efficient detection of the targets in 10% whole blood. Thus, the sensitivity of virus detection in crude blood samples was not compromised by skipping the DNA purification. In the case of the mousepox virus, the detection level was less than 10 copies or 5 pfu/ml. In comparative reactions, commercial Taqs failed to amplify the targets in the blood samples. We also developed a novel protocol for real-time PCR virus detection from blood samples. The novel mutant enzymes should simplify, speed-up, and lower the cost of important clinical and diagnostic tests.

89A

A New Mycobacterium Species Causing Diffuse Lepromatous Leprosy

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Background: Mycobacterium leprae causes leprosy, a chronic disease that has plagued humans for millennia. Thus far, M. leprae strains collected worldwide are found to be genetically clonal, which hardly explains, on an etiologic basis, the varying clinical features and severity of the disease. We have discovered a new Mycobacterium species from three patients who died of the severest and distinct form - diffuse lepromatous leprosy, that is characterized clinically by diffuse non-nodular cutaneous infiltration and pathologically by endothelial invasion by the mycobacterium accompanied by endothelial proliferation, vascular occlusion, and/or vasculitis. Methods: The mycobacterium was purified from heavily infected, freshly frozen autopsy liver tissue followed by DNA extraction in one case. Paraffin-embedded skin tissue was used for DNA extraction in three cases. Six genes of the organism were amplified by polymerase chain reactions, sequenced directly or upon cloning, and analyzed. Findings: Remarkable genetic differences with M. leprae were found, including a 2.1% divergence of the highly conserved 16S rRNA gene that also contained a unique 19-base-pair sequence, and 6-14% mismatches among five other less conserved genes. Phylogenetic analyses of the genes of 16S rRNA, rpoB, and hsp65 indicated that the two most related organisms evolved from a common ancestor that had branched from other mycobacteria. Interpretation: These results, along with the unique clinicopathologic features of diffuse lepromatous leprosy, lead us to propose Mycobacterium lepromatosis species novum. This species may account for some of the clinical and geographical variability of leprosy. This finding may have implications for the research and diagnosis of leprosy.

90B

Detection of toxigenic C. difficile and VRE in faecal samples by rapid extraction and real-time PCR

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OBJECTIVE: Conventional procedures to detect C. difficile and VRE in stool samples suffer from low sensitivity or, alternatively, long turn-around times, and this prompted us to develop a molecular-based approach to detect these organisms. METHODS: PCR primers and probes were either designed (VanA, VanB) or adapted from the literature (tcdB), and a rapid crude extraction procedure and internal control were developed and optimized. The procedure was designed to allow a single extract to be used for both tests using separate PCR reactions. The VRE test was also developed and evaluated for swabs using a similar extraction method. The procedure was evaluated in comparison to culture (VRE) and to a commercial EIA for toxin A and B (C. difficile). 132 patient samples (48 stools, 84 swabs) were tested for VRE and 614 stool samples were tested for C. difficile. Discrepant C. difficile results were resolved in a 405 sample subset by culture and/or direct cytotoxicity testing. RESULTS: All 20 VRE-culture-positive swabs were positive for VanA by PCR and 22/24 positive stools were positive by PCR; no VRE-culture-negative stool or swab specimen was positive for VanA. No VanB-culture-positive patient samples were available, however both of 2 VanB isolates were positive by VanB PCR, and 129 of the 132 VanB-negative patient samples were negative by VanB-PCR. For C. difficile, 95% of EIA-positive samples were PCR-positive and 93% of EIA-negative samples were PCR-negative. For those discrepant samples systematically analyzed, 18/20 EIA-neg/PCR-positive samples were concluded to be true positives, and 2/3 EIA-pos/PCR-negative samples were considered true negatives. The inhibition rate was approximately 6%, and most of these results were resolved upon re-extraction. The reagent costs were approximately CA$2 per test, and combined labour for the dual test was approximately 5 minutes per sample (for runs of 20). CONCLUSIONS: The PCR method proved to be rapid, sensitive, and cost-effective. The C. difficile PCR test has substantially higher sensitivity and comparable specificity relative to EIA, and the VRE test has comparable sensitivity and specificity with greatly improved turn-around time relative to culture. Van B positivity in VRE-negative specimens (in our case, in 2% of cases), has been noted before, and may require follow-up by culture.

91C

Genomics-Based Methods for the Detection and Whole-Genome Sequencing of an Unknown Virus

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Background: Conventional methods such as virus culture, electron microscopy, serology and PCR inherently fail to detect novel and uncommon viruses in clinical samples. Genomics-based methods such as high density DNA microarrays or shotgun sequencing are better suited for the detection of these
unknown viruses. **Objective:** A stool sample from a 6-year-old female with gastroenteritis was negative for norovirus by RT-PCR and negative for adenovirus and rotavirus by EIA. Upon examination by electron microscopy, virus-like particles were observed. The objective of this study was to identify the potential viral pathogen through shotgun sequencing of the extracted nucleic acid. **Methods:** The sample was concentrated by ultracentrifugation, treated with DNase, and total nucleic acid was extracted. This was randomly reverse transcribed, amplified and cloned to create a random library. The library was subjected to high throughput sequencing. A single clone containing a 195-bp fragment with 93% sequence identity to human parechovirus-1 (HPeV-1) was identified. Using primers based on this sequence, a total of 1.8 kb of sequence was recovered from the library. The rest of the parechovirus genome (HPeV-BC1) was cloned and sequenced using RT-PCR with primers based on the HPeV-1 and HPeV-3 genomes. **Results:** The 7.3kb genome of HPeV-BC1 has 93% sequence identity to HPeV-1. Across the capsid region, HPeV-BC1 has 88-97% nucleotide identity to closely-related human parechoviruses. The 5’ UTR and 3’ UTR are highly conserved between HPeV-BC1 and other human parechoviruses. **Conclusion:** For clinical specimens in which viral pathogens are suspected but not detected by all available viral diagnostic tests, shotgun sequencing can be employed to identify unknown viruses. The random library can also be used for whole genome sequencing of any viruses that are detected.

92D

**Reversible efflux-mediated drug resistance of a natural product, NP304**

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Fungi have emerged as the fourth most common pathogens isolated in nosocomial bloodstream infections, nearly 40% of which prove fatal. Widespread and repeated use of azoles, particularly fluconazole, has led to the rapid development of azole resistance. Reduced intracellular accumulation of drugs is a prominent mechanism of resistance in Candida cells wherein genes encoding drug efflux pumps belonging to ABC (the ATP-binding cassette) and MFS (major facilitator) superfamilies of proteins are overexpressed. The ABC transporters, **CDR1, CDR2**, and an MFS pump **CaMDR1**, play a key role in azole resistance as deduced from their high level of expression found in majority azole-resistant clinical isolates. It is in high demand to discover reversible inhibitors for **CaMDR1**. We have developed a high throughput synergy screening (HTSS) strategy for novel antifungal agents. One hit NP304 was identified with specific activity to elevated **MDR1**. The results were confirmed under solid and liquid conditions with **MDR1** knockout clinical strains and its complementary strains, while the Fluconazole-susceptible wild-type strains could tolerate more NP304. Further studies demonstrated that the effects were attributed to more accumulation of NP304 when **MDR1** was overexpressed, which leads to apoptosis in fungal pathogens. SAR (structure-activity relationship) analysis further confirmed the results. The novel mechanism of NP304 would shed new lights for treating drug resistance in infectious diseases.

93A

**Controlling MRSA infection by screening for specific inhibitors to the pAba pathway**

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Serious epidemics of infectious diseases, such as MRSA (Methicillin-resistant Staphylococcus aureus) constantly remind us that we live in a universe of microbes, who evolves faster than the speed we develop anti-infective drugs. So attention was focused on searching for compounds with novel targets to tackle with microbial infection. The folic acid is essential for bacterial survival and the para-aminobenzoic acid (pAba) pathway which synthesizes the direct precursor of folic acid is limited in the prokaryotes, fungi, plants but not in vertebrates. And the enzymes in the pathway are ideal targets for the multi-resistant gram positive bacteria. Therefore the inhibitors to the pathway are expected to be excellent targets of selective antibacterial agents. We have built a high throughput screening model utilizing **Bacillus subtilis** as the test strain, which grew in the minimum medium with the presence and absence of pAba. Through screening of 7000 microbial extracts, SN-3 was identified with specific activity to the pAba pathway. It also showed high potency to MRSA. Its MIC to MRSA ranges from 0.0625 to 0.125µg/ml, about 12 fold better than that of vancomycin. SN-3 had no apparent toxicity as tested, indicating a potential novel drug candidate against multi-resistant gram positive bacteria.

94B

**A Rapid, Simplified Collection-to-Detection System for Typing and Subtyping Influenza Viruses Using Real-Time RT-PCR**

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Influenza type A (H3N2 and H1N1) and B viruses are the most prevalently circulating human strains. However, an increase in confirmed cases of high pathogenic H5N1 in humans has raised concerns of a pandemic underscoring the need for rapid, point-of-contact collection and detection. In this study, we describe development of a) 1 novel respiratory specimen collection solution and 2) highly sensitive and specific set of real-time RT-PCR (rRT-PCR) assays for type, i.e., influenza A and B, and subtype, i.e., H1, H3, and H5 detection of influenza viruses from clinical specimens. The sample collection reagent was developed and optimized for procuring high quality nucleic acids from clinical or environmental specimens, inactivating potentially infectious biological pathogens for safe transport of specimens, and stabilizing and preserving released RNA/DNA preventing hydrolysis/nuclease degradation for extended periods at ambient temperatures. These influenza primer and probes have been adapted for use in an optimized, all-inclusive thermostable reagent blend and can be utilized on several real-time PCR thermocyclers including field-deployable instruments. Using the H5-specific assay, the optimized reagent blend was stable at ambient temperature for 30 days and capable of detecting <10 viral copies. The RT-PCR reagents
are all-inclusive thus reducing possible contamination and abolishing the need for extensive reagent pipetting and ‘master mix’ preparation. The type A and B rRT-PCR assays detected all 16 (H1-H16) influenza A subtypes and both circulating B lineages (Yamagata and Victoria), respectively. In a family study, these assays detected influenza A from throat swabs and nasal washes and were more specific than antigen detection kits. The described specimen collection solution and stabilized influenza rRT-PCR assays could be useful for environmental, epidemiologic or point-of-care screening or during a pandemic.

95C

Blocking the quorum-sensing-dependent biofilm formation by Andrographolide Derivatives

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The available agents to treat bacterial infections today is restricted to antibiotics developed specifically to kill or stop the growth of individual bacteria. However, many pathogens grew in community called “biofilm”, which lose their sensitivity to antibiotics quickly. The observation that quorum sensing is linked to virulence factor production and biofilm formation suggests that many virulent organisms could potentially be rendered nonpathogenic by inhibition of their quorum-sensing systems. We developed an ultrasensitive bioassay to screen for the inhibitors of N-acyl-L-homoserine lactones (AHLs) pathways. The test strain was constructed in Agrobacterium tumefaciens by using the T7 expression system to overproduce the AHL receptor TraR. Through screening of our natural product library, novel analogues of Andrographolide were identified to inhibit quorum sensing, which also significantly reduced the production of virulence factors and biofilm formation of Pseudomonas aeruginosa. Those compounds may provide a means of treating many common and damaging chronic infections without the use of growth-inhibitory agents, such as antibiotics, preservatives, and disinfectants, that unavoidably select for resistant organisms.

96D

Understanding Current Laboratory Biosafety and Biosecurity Practices Around the World

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This presentation will discuss the results of a multipart study to survey scientists in Asia, Latin America, Eastern Europe, and the Middle East and Caspian Basin. This study was designed to identify whether and how the respondents ensure the safe and secure handling of the pathogens and toxins that they store, work with and/or ship. The presentation will highlight regional results, comparing

• Research objectives and capabilities,
• Risk perceptions,
• Laboratory biosafety and biosecurity implementation,
• Communication and collaboration, and
• Challenges to conducting research.

By analyzing this information, we can better manage gaps in the development and implementation of biosafety and biosecurity measures. The results of the surveys provide insights into designing regionally appropriate strategies to promote the biosafety and biosecurity measures recommended in internationally recognized documents, such as the World Health Organization’s documents, “Laboratory Biosafety Manual” and “Biorisk Management: Laboratory biosafety guidance.”

97A

Conventional and Molecular markers of Coagulase Negative Staphylococcus in Nosocomial Infections

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Introduction- Coagulase negative staphylococci(CONS) long regarded as non-pathogenic and dismissed as cultural contaminants have emerged as a major nosocomial pathogen associated with sepsis and nosocomial infections. With increasing use of prosthetic devices, intravascular catheters, & other invasive technologies their important role as a pathogen has been recognized in recent years.

Aim-The present study has been designed to evaluate the conventional and molecular markers of nosocomial CoNS isolates and whether we may rely on the presence or absence of certain genes being involved in the pathogenicity of CoNS

Material &Methods-In this study 50 nosocomial isolates of CONS, over a period of six months in a tertiary care hospital were evaluated. Of these 25 strains were clinically significant and 25 were saprophytic. The strains were collected from blood cultures in cases of sepsis, central venous catheters, deep wounds, urine samples and other sources. Tests for conventional markers of virulence like tube coagulase, DNase, phosphatase and slime (biofilm) production were undertaken to differentiate invasive strains from non-invasive ones. Novobiocin (5µg) susceptibility test was performed to differentiate between S. epidermidis & S. saprophyticus. Multiplex PCR was used to explore the atlE and ica genes, as molecular markers to determine pathogenicity since they are involved in initial adherence, intercellular adhesion and mediate the formation of biofilm. The mecA gene was also evaluated since it controls the synthesis of the additional penicillin binding protein PBP2 in methicillin resistant staphylococci, commonly seen in CONS.

Results-In this study two genotypic markers discriminated between infectious & skin flora strains, namely, presence of ica operon & mecA gene. The ica operon was present in 12 of 25 infectious strains (48%) & in none of 25 skin flora strains. The mecA gene was present in 23 of 25 infectious strains (92%) & in only 2 of 25 skin flora strains (8%). Biofilm was formed by 23 of 25 infectious strains (92%) but by only 1 of the skin flora strain. The mecA gene was present in 13 of 15 strains (86%) isolated from OPD cases. The ica operon was present in 9 of 15 strains (60%) isolated from OPD cases. All 8 strains isolated from wards, had mecA gene but only one (12.5%) had ica gene. Two strains were isolated from ICU & both had
mecA & ica genes. **Conclusion:** This study demonstrates the ability of detecting ica & mecA gene loci and biofilm production to discriminate between contaminating & infecting CoNS. The ica and mecA genes were detected in significant number of pathogenic strains to suggest their relationship as potential virulence markers.

98B

**Development of a Portable PCR System Utilizing Automated Sample Preparation, Amplification, and Analysis**

**J. Czajka:** Smiths Detection, Edgewood, MD

For a variety of applications the ability to detect and confidently identify infectious agents near the source or patient would provide extensive benefits, which include improved treatment and disease containment. The use of molecular diagnostics for near-patient or remote testing has been limited to-date due to the complex sample preparation requirements and limited portability of the instrumentation. Portable PCR testing has relevance in many fields, including the detection of biological warfare agents in the security sector (bioterrorism), infectious agents detection for veterinary applications (livestock and herd management), and for clinical application (near-patient, or point-of-care). The boundaries between these applications blur, when considering infectious agents known to be, or which could be, transmissible between species, or which can be readily spread by contact between animals or people.

Smiths Detection has developed a field-portable platform for biological detection of such biological threats, the BioSeeq® PCR system. The BioSeeq® system provides the ability to collect, process, detect, and identify infectious diseases, through the use of unique nucleic acid isolation, amplification, and hybridization technologies. The instrument platform is a ruggedized, portable, battery operated, fluorescence thermocycler, with sensitivity of detection comparable to laboratory-based systems. The device has multiple independently controlled thermocyclers, allowing for different samples and assays to be run simultaneously, and the device is water-tight, which allows for decontamination at point of use by complete submersion. The sample preparation and assay reagents are contained in a single disposable consumable that, like the instrument can be decontaminated by submersion. The process of sample preparation is controlled and driven by the instrument which manages the purification and amplification of target nucleic acids from samples that range from simple powders (white powder incidents) to highly complex biological matrices. The system also utilizes a novel amplification methodology known as LAITE-PCR, that enables multiplexed detection of 10 - 20 target organisms in a single sample reaction. The BioSeeq® system has been developed to provide the capability of in-the-field or point-of-care PCR testing. The system does not require molecular biology expertise and is as simple as sample in, answer out. This system will provide improved diagnostic capabilities by decreasing the time to result and improving the detection and identification infectious agents, hopefully reducing the morbidity and mortality associated with many diseases.

99C

**ADENOVIRUS 14 DETECTION AND SURVEILLANCE IN AN OREGON HEALTH SYSTEM FROM 2005-2007**

**C. A. Gleaves:** Providence Medical Center, Portland, OR

**Background:** Adenoviruses are a common cause of mild respiratory illness, but may be associated with more severe disease in patients with compromised immune systems. There are 49 different types of Adenovirus that cause infection in humans. A cluster of community-acquired Adenovirus pneumonia, with 20% mortality, in otherwise healthy adults was reported at Providence Medical Center in early 2007. Subtyping of the virus strains from this outbreak was performed by PCR and identified Adenovirus 14 as the etiology. The Ad14 strain was first recognized in 1955 and has been reported rarely since. This study utilized banked specimens from 2005 to the present to determine the prevalence of adenovirus 14 infections in the Providence Oregon pediatric and adult populations.

**Methods:** Samples submitted in M4 or M4RT viral transport media and testing positive for Adenovirus by PCR were stored frozen at -20°C or below. All samples were extracted on the Roche MagNA Pure LC or BioMerieux easyMAG instrument. Ad14 real time PCR was performed in 96-well plates with the ABI Prism and ABI 7000, and carried out to 40 cycles, using sequences provided by the Center for Disease Control. 25 of 43 samples collected in 2005, 30 of 53 samples collected in 2006, and 40 of 46 positive samples collected in 2007 were tested for Ad 14 real time PCR. **Results:** 15 of the samples collected in 2005, 17 of the samples collected in 2006 and 20 of the samples collected in 2007 were on patients over the age of 18. Three of 25 Adenovirus positive samples (12%) collected in 2005 tested positive for Ad14. Of these, 100% were collected from patients over 18 years of age. 14 of 30 Adenovirus positive samples (50%) collected in 2006 tested positive for Ad14. Of these, 100% were collected from patients over 18 years of age. 26 of 40 Adenovirus positive samples (65%) collected in 2007 tested positive for Ad14. Of these, 19 (73%) were collected from patients over 18 years of age. **Conclusion:** Based on this retrospective study, the prevalence of Ad14 in the Providence Health System Oregon patient population has increased over the last three years. Ad14 prevalence is also increasing in the pediatric population. Active prospective surveillance for Adenovirus 14 is planned for 2008.

**100D**

**Analysis of resistance mechanisms in Pseudomonas aeruginosa from pediatric patients**

**D. Fang:** Beijing Children’s Hospital Affiliated to Capital University of Medical Sciences, Beijing, CHINA

**Background** In the present study, we characterized multidrug-resistant *Pseudomonas aeruginosa* (MDRP) clinical isolates from a pediatric facility, and investigated the types and features of the metallo-β-lactamases (MBLs) produced by carbapenem-resistant strains. **Methods** In all, 498 strains of *Pseudomonas aeruginosa* were isolated from patients at Beijing Children’s Hospital between January 2005 and December 2006. The MICs
372 women attending routine cytology Thin-Prep screening were tested for the presence of HPV infection in a private laboratory in Hong Kong. The age range of this group of women was 12 to 61 years old (mean 37.4, median 37.0). The cytologic diagnoses were normal (N=165), inflammation (N=54), infection (N=23), ASCUS (N=102), ASCUS-H (N=2), AGUS (N=1), LSIL (N=23) and HSIL (N=2). Using PCR with the general primers GP5+/6+ and SPF1/2 and type specific primers for the detection of HPV type 6, 11 (low risk) and 16, 18, 31, 33 (high risk), a total of 184 samples were tested HPV positive (49.5%). The low risk types 6 and 11 constituted 14 (7.6%) and 9 (4.9%) cases respectively, while the high risk types 16, 18, 31 and 33 were present in 12 (6.5%), 15 (8.2%), 10 (5.4%) and 10(5.4%) respectively. 8 (4.4%) cases had more than one type of HPV and all these cases harbored at least 1 high risk HPV type. In regard to the presence of HPV in various cytologic diagnoses, HPV was present in 27.3% in normal women, 42.6% in patients with inflammation, 52.2% with infection, and 77.5% in ASCUS, 100% in ASCUS-H and AGUS, 87% in LSIL and 100% in HSIL. These figures of HPV positivity were far greater than previously local studies in the general population. It would be interesting to see if future studies on HPV vaccination may be helpful to lower the prevalence of HPV infection in women of the general population in Hong Kong.
103C

Molecular Diagnosis of Mosquito Borne Diseases Panel commonly observed in India

D. Darves, S. Basak, D. Saranath; Relainace Life Sciences, Navi Mumbai, INDIA

Malaria, dengue fever and chikungunya fever are relatively high incidence mosquito borne diseases in the Indian scene. Diagnostic tests to confirm the diseases are serology based, and in case of malaria the signet shaped red blood cells indicative of malaria. These tests exhibit low sensitivity and/or a longer window period. Polymerase chain reaction (PCR) based molecular diagnosis with detection and identification of the etiologic agents is considered the method of choice, with high specificity and sensitivity of the assay. At Molecular Diagnostics and Genetics, Reliance Life Sciences, we have developed and validated in-house PCR based assays for detection of common mosquito borne diseases with specificity and sensitivity >95%, at a cost effective price. The diagnosis for malarial parasites involves nested-PCR to amplify the 18S rRNA of Plasmodium species, followed by direct sequencing of PCR products. We observed 70% positivity in the referral samples, with a higher prevalence of plasmodium vivax and Plasmodium falciparum. Clinical profile of chikungunya fever closely resembles dengue fever. Hence, it is imperative to unequivocally diagnose the etiologic agent, for appropriate patient management. Nested RT-PCR protocols with >95% sensitivity and specificity, in-house developed and performance characteristics validated targeting E1 region of Chikungunya viral genome, and the polyprotein gene region of the Dengue virus. The dengue virus were typed into type 1, 2, 3 and 4 by direct use of species specific primers for the viral typing. We observed presence of Chikungunya virus in 16% of the referred samples. Whereas, the Dengue viral types showed the presence of 484 bp, 119 bp, 290 bp, and 392 bp PCR products indicated Dengue type 1, 2, 3, and 4 respectively. We observed presence of Dengue virus in 76 of 227 (33%) referral cases, with majority (91%) Dengue virus type 3, and about 3% each of type 1, 2 and 4. Thus, we have demonstrated the accuracy, specificity and sensitivity of PCR based testing as a rapid, cost effective mode of detection and identification of etiologic agents.

104D

RET GENE MUTATIONS IN INDIAN PATIENTS WITH MEDULLARY THYROID CARCINOMA

B. Sharma, D. Saranath; Relainace Life Sciences, Navi Mumbai, INDIA

Medullary thyroid carcinoma (MTC), accounts for 3-10% of all thyroid carcinoma and 13.4 % of deaths due to the disease. Familial MTC (FMTC), Multiple Endocrine Neoplasia MEN 2A and MEN 2B, comprise the hereditary forms of MTC and constitute 25% of MTCs. MTC is inherited and shows autosomal dominant penetrance. Several germline mutations in the RET proto-oncogene, predisposes individuals to MTC. The aim of the current study is to determine frequency and position of the germ-line mutations in RET gene associated with familial MTC in the Indian group of patients. High-risk mutations in exons 10, 11, 13, 14, 15 and 16 of RET gene were examined for mutations and genotype-phenotype correlations. The protocol of analysis involved DNA extraction from blood samples of 52 MTC patients and family members and 40 normal individuals with no family history of MTC. The DNAs were subject to PCR amplification of the RET gene exons, followed by direct nucleotide sequencing of the PCR products using automated nucleotide sequencer Genetic Analyser 3100. MTC patients and high-risk individuals were referred for RET gene mutations by endocrinologists and oncologists. Of the 52 samples, 20 (38%) samples demonstrated mutations, primarily in Exon 11, codon 634. The mutations associated with MEN2A and confirmed the clinical symptoms as diagnostic of MEN2A. The mutations in 15 samples demonstrated Cys 634 Arg, as the most prevailing mutation in our patients. Two samples demonstrated Cys 634 Tyr, and a single sample showed Cys 634 Gyl. The other mutations identified in one patient each, were in Exon 10, codon 618 - Cys to Gyl confirming MEN2A symptoms, and Exon 16, codon 918 - Met to Thr, identified as MEN2B. A common non-synonymous polymorphism at Exon 11, codon 691 - Gly to Ser, was observed in 10.5% - 7 of 67 samples examined for RET gene. The samples constituted a total of 37 individuals with family members from 9 families, varying from 2 - 8 members, including the nine index cases with symptomatic MTCs. In four families, neither the patient nor any of the siblings showed the RET gene mutations, indicating spontaneous MTC in the index case. Of the five families with 2 to 8 siblings, five patients and 10 of 14 siblings showed the codon 634 mutation. One additional index case showed Exon 10, codon 618 mutation, not observed in the sibling. Forty control normal samples showed no mutations in the RET gene. Thus, our results indicate the utility of RET gene mutations as a diagnostic test in hereditary and spontaneous MTC patients of Indian ethnicity, as also early identification of the associated risk of development of MEN2A/MEN2B /FMTC in the siblings showing the mutations.

105A

Cost effective, Rapid, Sensitive HIV Proviral DNA Assay for Virus Detection in Infants and HIV Real Time PCR for Viral Load Determination

B. Sharma, A. Seth, K. Deshmane, S. Iyer; D. Saranath; Relainace Life Sciences, Navi Mumbai, INDIA

The Joint United Nations Program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO), estimate 2.5 million Indians, including 70,000 children, infected with the human immunodeficiency virus (HIV). The diagnosis of pediatric HIV-infection is complicated by the presence of transplacentally acquired maternal antibody, which usually persists in the infant for a year. ELISA and Western Blot are commonly used laboratory tests to diagnose HIV-infection in adults. However, these antibody-based tests do not differentiate HIV infection and passive transplacental transmission of HIV antibodies. Diagnosis of HIV-infection in infants up to 18 months of age necessitates detection of HIV particles. We have developed a nested PCR amplification targeting the LTR-gag region of the integrated HIV genome to detect HIV proviral DNA. A Real Time PCR assay using Taqman technology to detect and/or quantitate viral load in plasma. A five point ten-fold serial dilu-
Emerging Technologies of Medical Importance

S. Iyer, S. Basak, H. Patil, D. Saranath; Relainace Life Sciences, Navi Mumbai, INDIA

Hepatitis C Virus (HCV) has been identified as a major cause of chronic liver disease leading to cirrhosis of liver and hepatocellular carcinoma. The current global scenario estimates 170-200 million individuals infected with HCV, with the burden in India being 12-13 million. The HCV genome demonstrates a high degree of heterogeneity, leading to the definition of six different genotypes and several subtypes. The prevalence and distribution of HCV genotypes depends on geographic location, often associated with endemcity of the genotype. HCV genotype identification is critical for a patient/carrier as it indicates responsiveness to specific drugs. Identification of HCV genotype is also an epidemiological marker to trace the source of HCV infection in a given population. A comprehensive study of HCV genotype in Indian cohort of 1944 patients was undertaken at our laboratory. PCR amplification of the core-envelope region and 5' UTR was used to detect HCV, followed by nucleotide sequencing of the core-envelope/5'UTR region, using forward and reverse primers, on a ABI 3100 Genetic Analyzer. Nucleotide sequencing is the most reliable and unequivocal method, considered as a 'gold standard' for virus genotyping. The test was developed and its performance characteristics including validation of the test were determined at our laboratory. Ninety-two serial samples, developed and validated in-house, is an excellent indicator of efficacy of treatment in HCV patients.

106B

High Proportion of Hepatitis C Virus Genotype 3 in a Large Cohort of Patients from India

Hepatitis C virus (HCV) has been identified as a major cause of chronic liver disease worldwide, with an estimated 42 million HCV carriers in India. Chronic HCV infection may progress to liver cirrhosis and hepatocellular carcinoma in 3% of HCV infected individuals. The treatment of choice in HCV infected patients is long-term use of antivirals, which may induce drug resistance due to mutations in the viral genome. Mutations in the reverse transcriptase polymerase gene of HCV, confers resistance to antiviral treatment. With a view to providing a cost effective, rapid, specific and sensitive test to determine HCV drug resistance, we have designed a PCR based test, followed by nucleotide sequencing of the PCR product, with forward and reverse primers, to detect eleven different mutations in the Pol gene associated with antiviral resistance to several commonly used drugs i.e Lamivudine, Adefovir and Entecavir. The test was developed and its performance characteristics including validation of the test were determined at our laboratory. Ninety-two referral samples were analyzed for detection of drug resistance related genetic mutations. Sixty samples (65%) were detected as HCV positive using PreS and PreX region PCR. Seventeen (29%) samples were HCV negative, the lower limit of virus detection of our test being 100 copies per ml of serum or plasma. We observed 5/92 (6%) samples, did not amplify in the Pol gene, although showed consistent amplification in the PreS/PreX region, implying presence of HCV. The PCR assay demonstrated 93% sensitivity and 100% specificity. Among the HCV Pol region PCR positive 60 samples, 25 (42%) patients demonstrated mutations depicting drug resistance, with a majority (80%) detected with Lamivudine resistance associated primary mutations, M204V/I/S and L180M/V/I. Adefovir associated primary mutations, A181T and N236T, were detected in 10% cases. A single patient showed multiple drug, Lamivudine and Entecavi (M250V), resistance associated mutations. The results highlight the high incidence of drug resistance due to drug induced mutations in HCV, and indicate the importance of testing for HCV resistance with implications in treatment regimen and prognosis of the patient.

107C

Drug Resistance Mutations Detection in Hepatitis B Virus by Nucleotide Sequencing Based Assay

S. Basak, S. Kulkarni, D. Saranath; Relainace Life Sciences, Navi Mumbai, INDIA

Hepatitis B virus (HBV) is one of the major causes of liver disease worldwide, with an estimated 42 million HBV carriers in India. Chronic HBV infection may progress to liver cirrhosis and hepatocellular carcinoma in 3% of HBV infected individuals. The treatment of choice in HBV infected patients is long-term use of antivirals, which may induce drug resistance due to mutations in the viral genome. Mutations in the reverse transcriptase polymerase gene of HBV, confers resistance to antiviral treatment. With a view to providing a cost effective, rapid, specific and sensitive test to determine HBV drug resistance, we have designed a PCR based test, followed by nucleotide sequencing of the PCR product, with forward and reverse primers, to detect eleven different mutations in the Pol gene associated with antiviral resistance to several commonly used drugs i.e Lamivudine, Adefovir and Entecavir. The test was developed and its performance characteristics including validation of the test were determined at our laboratory. Ninety-two referral samples were analyzed for detection of drug resistance related genetic mutations. Sixty samples (65%) were detected as HBV positive using PreS and PreX region PCR. Seventeen (29%) samples were HBV negative, the lower limit of virus detection of our test being 100 copies per ml of serum or plasma. We observed 5/92 (6%) samples, did not amplify in the Pol gene, although showed consistent amplification in the PreS/PreX region, implying presence of HBV. The PCR assay demonstrated 93% sensitivity and 100% specificity. Among the HBV Pol region PCR positive 60 samples, 25 (42%) patients demonstrated mutations depicting drug resistance, with a majority (80%) detected with Lamivudine resistance associated primary mutations, M204V/I/S and L180M/V/I. Adefovir associated primary mutations, A181T and N236T, were detected in 10% cases. A single patient showed multiple drug, Lamivudine and Entecavi (M250V), resistance associated mutations. The results highlight the high incidence of drug resistance due to drug induced mutations in HBV, and indicate the importance of testing for HBV resistance with implications in treatment regimen and prognosis of the patient.
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