ORAL PRESENTATIONS

MMicOr01 : **Synergistic lethality of influenza with bacterial infections - lessons from the 1918 and 2009 flu pandemics**

Keith Klugman	**Emory University; and MRC/Wits University**	**USA & South Africa**

Pneumonia is the leading infectious cause of death, yet little research is devoted to its treatment and prevention. Influenza may progress to bacterial pneumonia, but the extent of the contribution of bacteria to influenza associated pneumonia morbidity and mortality is unknown. Pneumococcal conjugate vaccine (PCV) was used as a probe to determine the role of the pneumococcus in super – infection of influenza associated pneumonia. In a double blind randomized trial of 9 – valent PCV it was shown that PCV prevented clinical pneumonia associated influenza by 45%. This suggests the majority of influenza associated pneumonias in children may be due to pneumococcal super-infection. An analysis of the contemporary literature of the 1918 influenza pandemic suggests, in contrast to prevailing wisdom, >80% of deaths occurred > 7 days after onset of symptoms (influenza resolved in < 6 days in > 90% of patients). In fact, the time to mortality of untreated pneumococcal pneumonia follows an identical time course to the 1918 influenza deaths. A recent autopsy recut of autopsy specimens from the epidemic found bacterial infection in all of the victims. In studies of blood cultures taken from living patients with pandemic influenza associated pneumonia in 1918, pneumococci were isolated in up to 50% of patients. A number of bacterial vaccine studies were performed during the 1918 pandemic and a re – analysis of these studies, using influenza cases as the denominator and using pneumonia and death as outcomes, suggest that pneumococcal killed bacterial vaccines may have prevented both pneumonia and deaths. Recent autopsy data from current H1N1 pandemic in the USA and Brazil as well as a study using massTag technology to identify pneumococcal carriage, suggest that a large fraction of hospitalizations and deaths in otherwise healthy individuals during 2009 may have been due to bacterial co – infection.

MMicOr02 : **Molecular techniques in surveillance**

Anne von Gottberg National Institute for Communicable Diseases

Molecular techniques have been used to improve both the sensitivity of diagnostic tests, enhance data of serotypes and serogroups, and also elucidate aspects of the epidemiology of disease by performing genotypic characterization. For example, the diagnosis of pneumococcal pneumonia is challenging, and the burden of this disease is largely underestimated. We aimed to determine the prevalence of pneumococcal DNAemia in patients presenting with severe acute respiratory infection. Blood pneumococcal bacterial loads were determined by quantitative real-time PCR

detecting lytA. A rapid and specific assay was developed for the diagnosis of pneumococcal pneumonia, with an 8-fold higher detection rate than blood culture. Peaks in pneumococcal detection rates correlated with peaks for seasonal and pandemic influenza supporting the synergistic interaction between these pathogens. Laboratory-confirmed invasive meningococcal disease is traditionally defined as isolation of Neisseria meningitidis from normally sterile site specimens. Prior to 2004, culture-negative specimens, but either antigen positive for N. meningitidis or presence of Gram-negative diplococci alone were excluded from our laboratory-based surveillance programme due to specificity concerns. Additionally, loss of isolate viability during transport resulted in loss of serogrouping data. We aimed to improve the detection and characterisation of N. meningitidis using molecular methods. PCR added significant value in improving number of cases with laboratory-confirmed invasive meningococcal disease. Real-time PCR increased the sensitivity of both organism detection and serogroup characterisation. Within our group molecular epidemiology has been used to describe fluoroquinolone- and erythromycin-resistance pneumococci in South Africa, to understand the clonality of increasing serogroup W135 meningococci during an outbreak in 2006, as well as to characterise penicillin non-susceptibility in meningococcal isolates causing invasive disease.

MMicOr03 : **The MALDI Biotyper as a new standard tool for microbial identification in research and routine analysis**

Wolfgang Pusch Bruker Daltonik GmbH Bremen Germany

In the last couple of years MALDI-TOF mass spectrometry based microbial identification has replaced classical phenotypical identification approaches in many research and routine laboratories in more than one way. This paradigm change was initiated in Germany and since has spread rapidly all over Europe in the last two years. In the meantime the technology also gets increasing attention in the US and in Asia/Pacific.
Such new technology, which starts with the claim to become the new gold standard for microbial identification purposes has to be 1) accurate, 2) fast, 3) cost-effective, 4) acceptable to the scientific community and regulatory agencies, 5) simple to operate, 6) amenable to training, 7) user-friendly concerning the necessary reagents, 8) represented by a company with a great reputation, 9) represented by a company with exceptional technical service and 10) low in laboratory space consumption.
This presentation will give an overview, how the MALDI Biotyper fits into these attributes of an ideal rapid test procedure. Moreover, applications in direct microbial identification out of urine and positive blood cultures will be presented.

MMicOr04 : **Automated Serology- Our experience in an integrated lab and 'on the track'**

Trusha Nana NHLS

A number of factors require careful consideration when introducing a new automated serological assay. Some of the important issues we have had to consider in adiition to the assay's performance characteristics, are the cost of the test and the possible need for a change in the current testing algorithm. The latter in particular, necessitated consultation with clinicians at our site. Our integrated (clinical pathology) automated lab offers a limited spectrum of automated serological assays (HIV and hepatitis A, B and C; with syphilis to follow soon). The initial HIV assay and the hepatitis assays are run on analyzers on the track. Transient problems were experienced during the first weeks following the opening of the autolab. However, subsequently the track has allowed for high throughput testing on site 24/7. There has been a concommitant reduction in staff hands-on-time, enabling us to more effectively utilize our skilled microbiology technologists.

MMicOr05 : **Cost effective diagnosis and management of CMV disease**

Marvin Hsiao University of Cape Town, National Health Laboratory Service

Cytomegalovirus is an opportunistic pathogen that causes various end organ diseases (EOD) in immunocompromised individuals. Infected individuals harbour latent CMV and shed it intermittently in bodily fluids. In sub-Saharan Africa where CMV infection is common, using serological markers of infection or markers of viral shedding to guide treatment can lead to significant over-treatment. Although tissue biopsy and histology remains the gold standard for diagnosis of many forms of CMV end organ disease, molecular assays such as PCR have a shorter turnaround time and require less invasive sampling procedures. For many “hard-to-biopsy” EOD’s such as CMV retinitis and CNS disease, qualitative CMV PCR is becoming the new gold standard in many laboratories.

Molecular assays are an expense that resource poor settings can ill afford. However, considering the high costs of hospitalisation and ganciclovir therapy, an assay which can reliably identify CMV disease can be cost effective. In the post transplant setting, for example, the pre-emptive treatment approach with serial monitoring of blood CMV viral load to identify early CMV disease reduces overall ganciclovir use.

In HIV infected infants, CMV plays an important pathogenic role in severe pneumonia. These infants are often critically ill and lung biopsy is usually not feasible. Quantitative CMV PCR in blood or respiratory tract samples has a high negative predictive value and can be used to guide clinical management of these infants.

MMicOr06 :

MMicOr07 : **Near-patient testing for virology: panacea or peril?**

Wolfgang Preiser Medical Virology, University of Stellenbosch

Near-patient tests, also known as point-of-care (POC) or bedside tests, can be performed outside a laboratory by various types of healthcare staff and typically give a result within 10 to 30 minutes. Their availability has revolutionised some areas of medicine: most HIV infections in developing countries, and increasingly also those in industrialised countries, are diagnosed through rapid HIV antibody tests. Their main advantage is the almost immediate availability of the result, avoiding the need for a second appointment which the patient may not attend.
However, not everything is well as regards rapid HIV testing. Despite clear guidance from WHO and others, choice and evaluation of assays, user training and supervision, ongoing quality assurance, and the quality and performance of the available assays themselves are often still problem areas in practice.
Rapid tests for other viral diseases, such as diarrhoea and respiratory tract infections, are increasingly becoming available. Although their potential advantages in guiding appropriate patient management including infection control from the start are considerable, they have so far disappointed in practice. Apart from the unresolved challenges around user skills and knowledge and quality control, it is mostly insufficient test performance in terms of sensitivity and / or specificity which has led to several recommendations against the use of such tests. The latest example is the H1N1(2009) "swine" influenza pandemic. Rapid influenza antigen tests would have been useful in many cases, however numerous studies found they did not perform well and thus made little contribution to dealing with the pandemic.

MMicOr08

MMicOr09

Changing the 'culture' of microbiological testing

Mark Nicol, University of Cape Town

The microbiology laboratory of today still relies largely on the use of culture-based, phenotypic methods for detection and identification of micro-organisms. Molecular testing is increasingly offered, but remains restricted to a few selected pathogens. Recent advances in automation of sample processing and molecular detection raise the possibility that culture-based methods will become less relevant in our laboratories. This will require, however, a change in the culture of both microbiologists and clinicians and may lead us to ask fundamental questions about the role of the clinical microbiology laboratory in patient management.

MMicOr10

MMicOr11

MMicOr12 : Molecular tools for identification of organisms

Warren Lowman NHLS SA 0114898577

Rapid, reliable and accurate identification of microbes is a critical aspect in the management of infectious diseases. Traditional phenotypic methods are neither rapid nor objective, relying heavily on growth of the organism and expression of variable

phenotypic traits. Use of the 16S rRNA gene as a means of identifying prokaryotes has revolutionized the taxonomy of bacteria and has highlighted the deficiencies of non-molecular tools. PCR and various forms of nucleic acid-based technology have seen widespread use in the field of infectious diseases, however the use of this technology for the identification of bacteria has seen limited application in routine clinical microbiology. The reasons for this are varied and require further discussion. I will explore the value and potential of molecular-based identification of bacterial pathogens, assess current and future promising methodologies available for this purpose, and highlight potential pitfalls associated with the introduction of this exciting technology into the field of bacterial identification.

MMicOr13 : **The essential minimum in microbiology testing**

Elizabeth Wasserman NHLS & Stellenbosch University

Introduction:
The advance of technology presents us with a broader range of laboratory tests to identify the presence and characteristics of bacterial pathogens in clinical specimens. However, demands to restrain cost of laboratory investigations call for a continuous re-evaluation of our test menus and methods. This poses a particular challenge in the context of a developing country. Available skill and infrastructure are some of the limitations that may influence the character and scope of the service that we are able to offer.
Method:
Relevance of microbiological investigation is examined against the burden of disease, a priority grading of pathogens as proposed by the WHO, and the feasibility of a syndromic approach in specific clinical situations. Factors impacting on the quality of service rendered by clinical laboratories are also taken into account.
Results:
A process to determine suitable test menus is proposed, primarily based on a match between the profiles of service to the disease profile of patients in a particular community. This results in a framework that offers different test for the diagnosis of bacterial disease in a system of tiered laboratory service.
Discussion:
The first step towards success of maintaining a network of functional high quality laboratories may depend on the relevance of the test menus. We have to balance what is needed to what can be done, and to that which we can afford. This demands consideration of the desired end-point of our laboratory investigations in different clinical settings.

MMicOr14
MMicOr15

POSTER PRESENTATIONS

MMicPost01 : **Significant disseminated infections with unusual morphology of Cryptococcus neoformans**

Khine Swe Swe/Han NHLS/UKZN DURBAN
Aarthi Singh NHLS/UKZN DURBAN
Dianithi Naidu NHLS/KZN DURBAN
Eshana Panday NHLS/KZN DURBAN
Cynthia Manickam NHLS/KZN DURBAN

Abstract

A 47 year old male HIV negative patient presented with pleural effusion and meningitis, who was admitted to Central referral hospital for further investigation and management. At the time of admission, he was on continuation phase of anti-Tuberculosis (TB) treatment for pulmonary TB that diagnosed at the peripheral hospital. The CSF, Blood culture, pleural fluid and bronchoalveolar lavage were sent to the laboratory for septic screen.
The Pleural fluid and bronchoalveolar lavage specimens were stained with Gram and India ink which showed confused different shape of morphology cells with thick capsules. After 48 hours, organisms grew on culture plates and identified as Cryptococcus neoformans by using API (D32C). It was confused to report as preliminary rapid result based on its characteristic capsulated morphology especially for HIV negative patient because of the Cryptococcal disseminated infections is recognised as common one of AIDS defining condition. We report a case of significant disseminated infections with unusual morphology of Cryptococcus neoformans.

Key wards: HIV, TB, thick capsules, preliminary rapid result, Cryptococcus neoforman.

MMicPost02 : **Investigation of MRSA -suspected outbreak at haemodialysis unit and peritoneal dialysis unit at Central Referable Hospital in Durban**

Khine Swe Swe/Han ***NHLS/UKZN***
Eshana Panday ***NHLS***
Y.M Coovadia ***NHLS/UKZN***
N.M. Phewa4, Janine D. Petzer5, Sibongile Mkize6. DOH

Introduction
The laboratory plays a vital role in confirmation of suspected outbreaks at hospitals for infection prevention and control purposes. MRSA is a common cause of numerous infections in hospital due to high selection pressure because of frequent use of

antibiotics and beneficial conditions for spreading through the hospital, for instance via hands of medical personnel (1).
Aim & Objectives:
To investigate the suspected MRSA outbreak based on high prevalence.
To investigate the possible source of MRSA infections at the units.
Materials and methods
Risk assessment was done in the units. The specimens from staff and environment were taken in Haemodialysis unit (HDU).
Only nasal swab of staff and patients were taken from peritoneal dialysis unit (PDU).
Identification of MRSA was done by using the laboratory SOP.
Results:
39 swabs, from HDU were received. MRSA were isolate from 8 swabs. There were three MRSA from nasal swabs, two from hands, and one each from BP cuff, curtain and air from dermatology unit. Three nurses were discovered to be carriers.
61 nasal swabs from PDU were received. Three patients and three nurses were carriers of MRSA. MSSA were isolated from nasal swabs of two patients and five nurses.
Conclusion AND Recommendation
There was no outbreak in the units. Dermatology patients mustn't be allocated in same unit with haemodialysis patients.Continuous in-service training, hand washing, environment cleaning must be done and advice to follow the MRSA protocol to eliminate the MRSA carriage. Vitek 2 system detected Mupirocin MIC of >8. It is suggested that Mu E test be done to detect the high level resistance routinely in the microbiology laboratory.

MMicPost03 : First detected isolate: GISA (Glycopeptides intermediate resistant Staphylococcus aureus) in a patient with recurrent bacteraemia at renal unit in KZN.

Khine Swe Swe-Han	**NHLS/UKZN, IALCH Academic complexMicrobiology Department**	**DURBAN**
Sarojini Govender	**Microbiology/ NHLS**	**DURBAN**
Pragashinee Pillay	**Microbiology/ NHLS**	**DURBAN**
MPMF da Silva	**Infection Prevention & control Department, NHLS/Johannesburg laboratory**	**Johannesburg**
Y M Coovadia	**NHLS/UKZN**	**DURBAN**

Introduction

Vancomycin- intermediate Stap aureus (VISA) isolates and GISA have been reported in several countries. In 1997, the first strain of GISA (reduced susceptibility to vancomycin and teicoplanin) was reported from Japan (1). Reliable detection of GISA

strains in clinical laboratories is critical. It is essential for appropriate treatment for the patients, infection prevention and control.

Material and methods
Three sets of Blood culture bottles with different time, pleural fluid and haemodialysis catheter tip were received from same patient who had chronic renal failure.
Vitek2 and CLSI Guideline were used for identification and susceptibility of Stap aureus.
CDC Guideline, E tests Macromethod and vancomycin screen plate (6.000 µg/ml), were used to detect the Heterogeneous hGISA and GISA.

Result
MRSA was isolated from all received specimens.
First isolate from blood culture specimens received was MRSA and MIC of Vancomycin was 2.
Second MRSA from second sent Blood culture and catheter tip were GISA (Vancomycin MIC= 6.000 µg/ml (4-8 µg/ml). Third MRSA from 3RD time blood culture was also GISA.
E test Macromethod (Screening test for heteroresistance to glycopeptides) results showed Vancomycin MIC: 12.000µg/ml and teicoplanin MIC: 12. 000µg/ml.

Conclusion
Patient was recovered (clinical and microbiologically responded) after 4weeks of linezolid that is appropriate drug therapy for GISA. Previously, his blood cultures were keeping positive while MRSA was treated with Vancomycin.
E test Macromethod should be done routinely if the Van MIC >1 (MIC 2 & >) MRSA isolate that was picked up by Vitek2.

MMicPost04 : **ACINETOBACTER BAUMANNII:An evaluation of five susceptibility test methods to detect tobramycin resistance in an epidemiologically related cluster.**

V. Mischka Moodley	**University Of Cape Town/ National Health Laboratory Services**
Stephen P. Oliver	**University of Cape Town/ National Health Laboratory Services**
Iva Shankland	**National Health Laboratory Services**
B. Gay Elisha	**University of Cape Town/ National Health Laboratory Services**

BACKGROUND
Acinetobacter baumannii is a major pathogen causing nosocomial infections, particularly in critically ill patients. Further, this organism has acquired the propensity to rapidly develop resistance to most antibiotics. At several academic hospitals within

Cape Town, tobramycin and colistin remain frequently the only therapeutic options. The Vitek2 automated susceptibility testing (AST) is used in the clinical laboratory to determine selected susceptibility profiles. The suspicion of a possible AST-related technical error when testing for susceptibility to tobramycin in A. baumannii precipitated this study.

METHOD
Forty A. baumannii strains obtained from clinical specimens (June-December 2006) were included in this prospective study. The strains selected exhibited MICs close to the tobramycin breakpoints (CLSI guidelines). The clinical isolates were predominately from intensive care unit patients (33/40) obtained from various sites of infection. AST was compared to disc diffusion, Epsilometer test and agar dilution using broth microdilution as the reference standard. Additionally, PCR was performed to detect aac(3)-II' gene which encodes an aminoglycoside modifying enzyme with activity against tobramycin. Molecular tools were utilised to study the relatedness of the isolates.

RESULTS
The tobramycin susceptibility results revealed errors in 25/39 isolates (10 very major and 15 minor errors) when AST was compared to broth microdilution (reference standard), 12/39 (1 very major and 11 minor errors) when Etest was compared to the reference standard, and 15 errors (3 very major and 12 minor errors) when disc diffusion was compared to the reference standard. The result for AST vs. the reference standard was statistically significant ($p<0.001$). Additionally, the tobramycin resistance gene, aac(3)-II,' was detected in 21/25 of the discrepant isolates, confirming the resistant phenotype detected by the reference standard. Molecular typing showed that these isolates were genetically related.

CONCLUSION
The Vitek2 automated tobramycin susceptibility testing should not be recommended for Acinetobacter baumannii.

MMicPost05 : **Phenotypic detection of carbapenemase production in Enterobacteriaceae: false positive result with the modified Hodge test**

Bheratkumar Bhagoobhai	***Dept of Clinical Microbiology & Infectious Diseases, Universi National Health Laboratory Services***
Lebogang Moreosele	***Dept of Clinical Microbiology & Infectious Diseases, University of the Witwatersrand and National Health Laboratory Services***
Ranmini Kularatne	***Dept of Clinical Microbiology & Infectious Diseases, University of the Witwatersrand and National Health Laboratory Services***

During the past decade Class A carbapenemase-producing Enterobacteriaceae have become a major global concern as widespread outbreaks have been increasingly reported. Early recognition of these carbapenemase producing pathogens is essential for the institution of appropriate antimicrobial therapy and for controlling their spread. In 2009, the CLSI published recommendations for the phenotypic screening and confirmation of carbapenemase production using the ertapenem or meropenem disc diffusion or broth microdilution test and the modified Hodge test (MHT), respectively.

We report a clinical Enterobacter cloacae isolate, that was identifed as a carbapenemase producer by a positive MHT. Initial automated identification and susceptibility testing (MicroScan negative breakpoint combo 43 panel), revealed a 99.9% ID probability with a constitutive AmpC hyperproducer phenotype. The isolate tested negative for extended-spectrum □-lactamase (ESBL) production using a triple combination disc method. The MicroScan ertapenem breakpoint of > 4 □g/ml, as well as a 10□g ertapenem disc zone diameter of 20mm, with discernible persisters within the inhibtion zone, were indicative of a potential carbapenemase producer. However, E-test MICs for all three carbapenems tested (ertapenem, imipenem & meropenem) revealed MICs in the low susceptibility range that were markedly below the CLSI screening cut-offs for carbapenemase production.

Conclusion: False positive MHT results have been described in Enterobacteriaceae strains which have increased baseline levels of resistance to carbapenems as a result of non-carbapenemase-mediated mechanisms. These include a combination of ESBLs or AmpC □-lactamases and porin mutations. The identification of a carbapenemase-producing isolate has significant clinical and public health implications. In the absence of readily applicable, cost effective molecular assays to establish the presence of carbapenemases, more accurate phenotypic confirmatory tests such as novel boronic acid based methods should be evaluated.

Categories: modified Hodge test, carbapenemases, antimicrobial susceptibility testing

Location: Microbiology laboratory, National Health Laboratory Services, Helen Joseph Hospital

MMicPost06 : Primary osseous echinococcosis: an unusual presentation of cystic hydatid disease

Lenel Jacobs	***Dept of Clinical Microbiology & Infectious Diseases, University of the Witwatersand & National Health Laboratory Services, Helen Joseph Hospital***
Ranmini Kularatne	***Dept of Clinical Microbiology & Infectious Diseases, University of the Witwatersand & National Health Laboratory Services, Helen Joseph Hospital***

The zoonotic parasitic cestode, Echinococcus granulosus, is highly endemic in certain regions of South Africa. Hydatid bone disease is a rare manifestation of infection, accounting for only 1.5 to 3% of all cases. Of all osteohydatid disease, 60% occur in the spine and pelvis; 28% in long bones; 8% in the ribs and 4% in the skull. Skeletal cysts are usually secondary to hepatic or pulmonary echinococcosis: however they may occasionally occur as a manifestation of primary bone disease.

We report a case of primary osseous cystic hydatid disease. The patient, a 30-year-old female, presented with a pathological fracture of the right radius after having fallen on her outstretched hand. Skeletal x-ray showed a transverse mid-diaphyseal fracture across an oval radioluscent area of bone. Surgical exploration revealed cortical destruction and soft tissue extension of a cystic mass. Gelatinous membranous material obtained during curettage of the cystic lesion was sent to the microbiology lab for routine microscopy and culture. Gram stain microscopy of the specimen revealed numerous protoscolices and hydatid hooklets. An indirect haemagglutination (IHA) test for the detection of serum antibodies to Echinococcus granulosus was negative.

Conclusion: The diagnosis of hydatid bone disease is primarily clinico-radiologic. However, radiographic signs in affected bone are non-specific and inconclusive. Hydatid serological tests lack sensitivity and specificity. Delayed or misdiagnosis may lead to complications such as disease recurrence, sepsis and limb amputation. We maintain that, in endemic regions, microbiological examination of material obtained from well-defined osteolytic lesions is invaluable in making a definitive diagnosis, and thereby improving the clinical outcome, of osseous echinococcosis.

MMicPost07 : Automated Low-Cost Fluorescence Microscope to Screen Sputum for Mycobacterium Tuberculosis

Sriram Krishnan	***University of Cape Town***	***South Africa***
Kenneth Tran	***University of Virginia***	***United States of America***
Broghan M. Cully	***Dartmouth College***	***United States of America***
Mahalingam Veeraragoo	***University of Cape Town***	***South Africa***
Dr. Genevieve Learmonth	***University of Cape Town***	***South Africa***
Dr. Tania Douglas	***University of Cape Town***	***South Africa***

Despite recent advances in diagnostic techniques for tuberculosis, microscope analysis of sputum smears continues to be the accepted procedure for screening, particularly in low and middle income countries. In these countries, fluorescence based smear microscopy is expected to be the primary screening tool for tuberculosis in the immediate future. Automated microscopy would enable laboratories in countries faced with a high incidence of tuberculosis to cope with the large volume of smears to be processed.

We have developed a fully automated low-cost fluorescence microscope built using off-the-shelf components. While automated slide scanning systems have mainly relied on attaching digital cameras to microscopes, our system replaces the traditional microscope by combining the necessary optical, mechanical and electronic components and a digital camera, and attaching them to a computer, for control of all aspects of slide analysis.

The system is currently being integrated with auto-focusing and image analysis algorithms that have previously been developed by us to process ZN-stained smears and which have been evaluated in our laboratory to yield acceptable results. The image quality from our fluorescence microscope is excellent and we are currently evaluating the overall system performance. Such an automated system would rapidly screen a larger portion of the slide than recommended by the WHO, potentially increasing overall sensitivity and specificity of screening. Thus it could replace manual microscopy in resource constrained settings.

MMicPost08 : Diverse norovirus genotypes identified in individuals affected by a multifactorial waterborne gastroenteritis outbreak in Mpumalanga, 2007.

Janet Mans **University of Pretoria**
Nicola A. Page **National Institute for Communicable Diseases**
Maureen B. Taylor **University of Pretoria**

Noroviruses (NoVs) are small, positive-sense RNA viruses that are implicated in outbreaks of non-bacterial gastroenteritis worldwide. NoVs infect persons of all ages and cause infections ranging from asymptomatic to acute vomiting and diarrhoea. They are shed in the stools of infected individuals, are highly infectious and are stable in the environment. Surface waters in rural and urban areas can be contaminated with human faecal matter. In 2007, from October through December, a large number of individuals in the Delmas area of Mpumalanga contracted diarrhoeal disease. Multiple pathogens (bacteria, parasites and enteric viruses) were detected in the majority of the diarrhoeal stool specimens submitted for analysis. Subsequently faecally contaminated drinking water was implicated as the source of the outbreak. Of 166 diarrhoeal stool specimens collected during the outbreak, 46 (28%) tested positive for NoV by real-time reverse transcription polymerase chain reaction (RT-PCR) (39/46) and/or enzyme immunoassay (7/46). NoV genogroup II predominated (22/46), with NoV genogroup I strains (15/46) and three mixed GI/GII infections being detected. Analysis of 22 of the NoV positive strains revealed a diverse range of types to be present in the patients. NoV was the only aetiological agent identified in 16 patients. NoVs from eight of these specimens were characterised and a single genotype was found in each specimen (I.1, I.5, I.6, I.14, II.1, II.2, II.9 and II.11). The results suggest that diverse NoV strains were circulating in the community at the time of the outbreak. The GII.4 strain, commonly implicated in NoV outbreaks where person-to-person spread is involved, was not detected which supports the idea that the NoV infections either originated from the contaminated water or were associated with sporadic NoV-associated gastroenteritis. In patients where, in addition to NoV, other

pathogens were detected, the NoVs could represent asymptomatic infections or mild infections contributing to the diarrhoea.

MMicPost09 : **Recurrent Streptococcal meningitis in HIV positive patients: a clinical & microbiological report of 2 cases**

Yesholata Mahabeer NHLS,Academic complex, Kwazulu Natal
Yacoob Coovadia NHLS, academic complex, Kwazulu Natal

We describe two HIV positive patients with recurrent Streptococcus pneumoniae meningitis.

PATIENT 1

A 27 year old male who was known to be HIV positive, was admitted to hospital on 26 July 2009 & 21 October 2009. On both admissions, he had meningitis caused by Streptococcus pneumoniae. His CD4 count was 119 cells/ul in October 2009. He demised after 15 days of ceftriaxone.

PATIENT 2:

3 yr old female admitted 29 December 2007 with a left sided pleural effusion on chest X ray. A pleural tap grew Streptococcus pneumoniae.

She was then admitted on 11 July 2009, 21 October 2009 & 10 March 2010 with repeat episodes of Streptococcus pneumoniae meningitis.

RESULTS:
TABLE 1:

RESULTS FOR PATIENT 1

Date	26/7/09	21/10/09		
Protein (g/L)	2,64	N/A	9,41	
Glucose (mmol/L)	0,4	N/A	0,2	
Polymorphs	882	N/A	29 600	
Lymphocytes	258	N/A	16 000	
Serotype	7C	28	ND	
Penicillin MIC (mg/L)		0,016	0,012	0,032

TABLE 2:

RESULTS FOR PATIENT 2

Date	29/12/07	11/7/09	17/7/09	24/7/09	21/10/09
	23/10/09	10/3/10			

Specimen	Pleural fluid		CSF	CSF	CSF	Blood culture		CSF	CSF
Protein	5,28	1,49	1,13	N/A	7,01	2,79			
Glucose		0,3	2,9	2,2	N/A	0,2	0,3		
Polymorphs		1202	50	60	N/A	286	100	234	
Lymphocytes		70	170	40	N/A	280	146		
Serotype	6A	4	N/A	N/A	4	ND	19A		
Penicillin MIC	ND	0,012	N/A	N/A	0,012	0,012	ND		

ND = Not Done
N/A = Not Applicable

CONCLUSION:
These two cases illustrate that Streptococcus pneumoniae meningitis can be recurrent in HIV positive patients. The repeat episodes were also mostly due to different serotypes. Although infections with this organism are common in this population, there are few reports in the literature that describe clinical & laboratory details of patients with recurrent pneumococcal meningitis in the HIV infected individual.

MMicPost10 : **Unusual Case of Cryptococcal Arthritis , Osteomyelitis and Cutaneous Involvement**

Dr Prasha Mahabeer Dept of Microbiology,NHLS IALCH KZN
Dianithi Naidu Dept of Microbiology, NHLS IALCH KZN
Dr M Archary Dept of Paediatrics, King Edward VIII Hospital KZN
Prof Y Coovadia Dept of Microbiology, NHLS IALCH KZN

A 5 year old child presented with disseminated cryptococcal lesions involving bone, joints and skin. Despite prolonged and appropriate anti-fungal treatment he continued to present with persistant and new cryptococcal lesions

Case History:
A 5 year old male child was admitted to King Edward Hospital in January 2010 with a history of generalized seizures. He was RVD positive ,WHO stage 4 on HAART since August 2009.
On examination he was found to be wasted and stunted with evidence of chronic lung disease.. He had generalized lymphadenopathy and multiple pale nodules and plaques on the face and body. He had a 5cm hepatomegaly and splenomegaly respectively, but no signs of meningism.
He remained pyrexial and was treated with intravenous antibiotics. A week after admission, swelling of his right ankle was noted. Fluid aspirated from the joint showed a yeast on gram stain. Cryptococcus neoformans was cultured and the patient was commenced on Amphotericin B.

Lytic bone lesions were also noted on the right tibia and a bone biopsy cultured Cryptococcus neoformans. Lumbar puncture revealed a normal CSF with a negative cultures.
Despite Amphotericin B, he continued to develop multiple abscesses on his scalp and a biopsy cultured C.neoformans.. The addition of Fluconazole also failed to prevent development of new lesions. Attempts to get 5- Flucytosine were unsuccessful.
Conclusion:
Cryptococcus is a common cause of meningitis in AIDS patients. Skeletal involvement occurs in 5-10% , with cryptococcal osteomyelitis being uncommon and arthritis rare.(1)
Cryptococcal disease as part of IRIS has been described .We report an unusual case of disseminated cryptococcosis refractory to antifungal therapy.

MMicPost11 : Diagnosis of active Tuberculosis using flow cytometry in HIV infected individuals in a Tuberculosis (TB) endemic setting: Optimisation of the assay

Suvarna Buldeo ***NHLS/Wits*** ***South Africa***
David Murdoch ***Duke Medical School*** ***USA***
Wendy Steven ***NHLS/Wits*** ***South Africa***
Melinda Suchard ***NHLS/Wits*** ***South Africa***

Introduction
Tuberculosis is one of the great killers despite adequate chemotherapeutic agents and a vaccine. The increased burden of the disease in South Africa is fuelled by the prevailing HIV endemic. HIV positive patients present atypically and are often sputum AFB negative.These patients are not only at increased risk of mortality from undiagnosed TB but they also serve as a point source for further spread of the disease. Due to the atypical presentation of tuberculosis in HIV positive people, current diagnostic assays are largely ineffective. We are exploring the use of flow cytometry to assist with the diagnosis of active tuberculosis in this population. We plan on detecting interferon gamma production from CD4 memory cells in the blood and sputum of suspected patients in response to two TB antigens, PPD and ESAT6 (Statens serum institute, Denmark). This assay has the ability to differentiate active and latent tuberculosis.

Method and Results
We have designed and optimised a simple functional four colour flow cytometric panel that identifies interferon gamma production from memory CD4 T cells (CD3+/CD4+/CD27-/IFNG+) in induced sputum following stimulation with the TB antigens PPD and ESAT6. We have shown that good quality sputum samples can be analysed flow cytometrically, showing stimulated cytokine production similar to that of peripheral blood mononuclear cells.

Discussion

Flow cytometry has the advantage over existing immunological assays such as EliSpot as it is a more flexible testing platform which enables the detection of the cells responsible for cytokine production in addition to quantification of the cytokine production. The immune response to Tuberculosis may be compartmentalized, with differing responses in blood and lung. We plan to use this assay to compare flow cytometric analysis of induced sputa samples with that of peripheral blood as a TB diagnostic tool in a high prevalence TB and HIV setting.

MMicPost12 : **Evaluation of the Tube Coagulase Test as a rapid method for detecting Staphylococcus aureus directly from positive blood culture bottles**

Dr. Rena Hoffmann	**Medical Microbiology, NHLS and University of Stellenbosch**
Mr. Larry May	**Medical Microbiology, University of Stellenbosch**

Introduction:

The ability to rapidly distinguish between the presence of S.aureus and coagulase-negative staphylococci (CNS) is essential for optimal patient management. The Tube Coagulase Test (TCT) is the gold standard for S.aureus identification and is performed on an overnight culture. Data suggest that the TCT can be performed directly from positive blood culture bottles and interpreted earlier than the standard 24-hour turnaround time.

Aim of the study:

To evaluate the TCT as a rapid, accurate and cost effective method for detecting S.aureus directly from blood cultures.

Method:

The dTCT test was performed over a 6-month period at Tygerberg Hospital on all positive blood cultures that showed a pure growth of gram positive cocci in clusters. The results were compared with the conventional methods used to identify staphylococci from overnight cultures.

Five drops of blood from a positive blood culture bottle (BACTECTM 9120 Standerd10 Aerobic/F) were added to a plastic test tube containing 0.5ml of rabbit plasma and shaken. Tubes were incubated at 35˚C aerobically and read at 2, 4 and 6 hours. The presence of a soft gel or clot that could not be resuspended by gentle shaking indicated the presence of S.aureus.

Results:

The dTCT was determined for 177 blood cultures with sensitivities of 8%, 38% and 40% at 2, 4 and 6 hours, respectively and a specificity of 100% throughout. No major cost benefit was shown when compared to existing test methods used.

Conclusion:

Due to the low sensitivity of the dTCT in our setting, this test can not be recommended as a rapid method for detecting S.aureus directly from blood cultures.

MMicPost13 : **Microbiology of wound infections following traditional circumcision in Mthatha, Eastern Cape, South Africa**

Sandeep Vasaikar Walter Sisulu University, Mthatha, ECP
Vivek Bhat Walter Sisulu University, Mthatha, ECP

BACKGROUND: The circumcision ceremony (initiation) in the Xhosa community of RSA marks the transition from boyhood to manhood and to honour the tradition of their forefathers. This is usually performed by experienced traditional practitioners assisted by traditional nurses. Unfortunately, in the past years young and inexperienced traditional surgeons have been conducting the ritual either openly or clandestinely.
Aims and objectives: to determine the frequency of various bacterial isolates from circumcision wounds prevalent in our setting and study their antimicrobial resistance patterns.
MATERIALS AND METHODS: This is a retrospective study of circumcision wound 338 isolates from 310 patients (multiple specimens from some patients) which were received by the Nelson Mandela Academic Hospital (NMAH) microbiology laboratory of the NHLS between January 2007 and April 2010. Specimens- from the NMAH, MGH and satellite clinics. All relevant specimens were collected as pus and pus swabs from the patients and processing of culture, identification and antimicrobial susceptibility testing was done by standard laboratory protocols.
RESULTS: A total of 293 patient specimens showed growth on culture. The total number of isolates was 586 out of which 226 were Gram positive cocci and 360 were Gram negative bacilli. The commonest organism was Staphylococcus aureus (20.3%), followed by Group D Streptococcus (17.2%), Proteus mirabilis (13.5%), Klebsiella pneumoniae (13%), Acinetobacter baumanii (9.7%), Pseudomonas aeruginosa and E. coli (5%). Generally high level of resistance was observed in many organisms. MRSAs 27.7%. Resistance among the GNB was, in general, least to imipenem, amikacin and cipro. Age varied from 15 to 28 years with most of patients (88.3%) from 15-21 years and 30.1% were below 18 years.
CONCLUSION: The common organisms causing circumcision wound infections in our setting include Staph, Group D Strept, Proteus, Klebsiella, Acinetobacter, Pseudomonas, E. coli and there is a high level of resistance against commonly used antimicrobials.

MMicPost14 : **High-Resolution Melt Analysis for the Detection of Mycobacterial Lymphadenitis Using Whatman FTA® Elute Cards**

Kim Hoek	**Division of Medical Microbiology, University of Stellenbosch/NHLS Tygerberg Hospital**
Colleen Wright	**Division of Anatomical Pathology, University of Stellenbosch/NHLS Tygerberg Hospital**
Ben Marais	**Department of Paediatrics and Child Health, University of Stellenbosch**
Paul van Helden	**Division of Molecular Biology and Human Genetics, University of Stellenbosch**
Robin Warren	**Division of Molecular Biology and Human Genetics, University of Stellenbosch**

The increased incidence of tuberculosis (TB) lymphadenitis due to the human immunodeficiency (HIV) epidemic and the significant diagnostic delay by routine culturing methods has led to the need for a more rapid diagnostic method. We recently reported a rapid and specific molecular-based method which consisted of high-resolution melting (HRM) analysis of fine needle aspiration biopsies (FNAB) inoculated into transport medium bottles and subsequently spotted onto FTA® Classic Cards (Whatman, UK). In the current study, we aimed to simplify the procedure by circumventing the need for a transport medium bottle and spotting the samples directly onto the simpler FTA® Elute Cards.

Fine-Needle Aspiration Biopsy samples were obtained from 65 adults and children with possible mycobacterial lymphadenitis and disease was confirmed in 41 patients (63.1%) according to the reference standard (positive cytology and/or a positive culture). Cytology was positive in 75.6% and culture in 80.5% of patients. HRM identified 56.1% (19/41) TB lymphadenitis samples with a sensitivity and specificity of 46.3% and 83.3% respectively as compared to the reference standard.

The use of the simpler more cost-effective FTA® Elute Card with the omission of the transport medium bottles did not provide an improvement over the previously described indirect method. However, both methods were able to provide a rapid and specific diagnosis in ~50% of patients, thereby allowing prompt initiation of therapy. The remainder of cases would be diagnosed at a later stage through routine phenotypic culturing methods.

We therefore propose a diagnostic algorithm which takes HRM and cytology results into consideration in that if HRM and cytology are indicative of mycobacterial disease, then treatment is initiated, if HRM and cytology do not correlate or are both negative for disease, then culture results are awaited before treatment initiation.

MMicPost15 : **Pooling strategies to reduce the cost of monitoring ART by HIV viral load testing**

Gert U. van Zyl	**Stellenbosch University and NHLS Tygerberg**	**South Africa**
Wolfgang Preiser	**Stellenbosch University and NHLS Tygerberg**	**South Africa**
Sussanne Potschka	**University of Würzburg**	**Germany**
Anna T Lundershausen	**University of Würzburg**	**Germany**
Richard Haubrich	**University of California, San Diego**	**USA**
David Smith	**University of California, San Diego**	**USA**

Background:
Quantitative HIV RNA ('viral load') testing surpasses CD4 count and clinical monitoring in detecting antiretroviral regimen failure; however its cost can be prohibitive. Recently, the use of pooling strategies with a clinically appropriate viral load threshold has been shown to be accurate and efficient for monitoring if virological failure occurs at a low rate. Clinical criteria might help to select patients with a low pre-test probability of failure for whom pooling would be appropriate.
Methods:
We used screening criteria (age above 15 years, on an NNRTI-based first-line regimen) and information provided on the routine test request forms of patient specimens that already been tested individually at Medical Virology, NHLS Tygerberg. Specimens from patients meeting these criteria and with sufficient sample volumes were included in the pooling study. Two different pooling strategies were compared: a minipool strategy, where every 5 specimens were tested together; and a matrix pool strategy where 100 specimens were tested in a 10x10 matrix resulting in 10 vertical and 10 horizontal pools. An algorithm was used to identify the positive specimen(s) from any pool with detectable HIV RNA. Investigators were blinded throughout to individual specimen test results.
Results:
Applying clinical criteria on the patient database resulted in a failure rate <10%. 370 patient specimens meeting the criteria were included. Pooling resulted in 34%-41% fewer HIV RNA tests having to be done. The matrix pool strategy had a higher efficiency but was significantly more labour-intensive and potentially error-prone.
Conclusions:
In resource-constrained settings, a combination of pre-selection of patients with low pre-test probability of treatment failure and pooled testing of their specimens can substantially reduce the cost of state-of-the-art monitoring of ART without compromising accuracy. This might allow resource-constrained countries to include viral load monitoring into their ART programmes.

MMicPost16 : **Antimicrobial susceptibility profiles of anaerobic bacteria isolated at Tygerberg and Groote Schuur Academic hospitals in the Western Cape**

H Orth **Medical Microbiology, Stellenbosch University & NHLS**
C Bamford **Medical Microbiology, UCT & NHLS**
R Hoffmann **Medical Microbiology, Stellenbosch University & NHLS**
C Lang **Medical Microbiology, NHLS Groote Schuur hospital**
J Goodway **Medical Microbiology, NHLS Tygerberg hospital**

Introduction:
Susceptibility testing of anaerobic organisms is not routine practice in clinical microbiology laboratories. Empiric therapy is based on resistance trends which are monitored through antimicrobial surveys. However, national and local surveys in South Africa have been infrequently done. International published reports of increased resistance among anaerobes, especially Bacteroides spp., prompted this local survey.
Methods:
Antibiotics used for empiric therapy of anaerobic infections at Tygerberg and Groote Schuur Academic hospitals and newer agents such as ertapenem and moxifloxacin were tested. Antimicrobial susceptibility testing was performed on 80 non-duplicate anaerobic clinical isolates identified by standard methods at the Medical Microbiology laboratories of above institutions during 2009. The MICs (minimum inhibitory concentrations) for the following 8 antimicrobial agents were determined by the E-test method: penicillin; co-amoxiclav; piperacillin-tazobactam; imipenem; metronidazole; clindamycin; moxifloxacin and ertapenem.
Results:
Over the study period 80 isolates were obtained of which the Bacteroides group was the most common (60%), followed by Peptostreptococcus spp. (10%). Piperacillin-tazobactam was the most active agent with 99% of all anaerobes testing susceptible, followed by ertapenem (98%), co-amoxiclav, imipenem (both 96%) and the new fluoroquinolone, moxifloxacin (95%).
Penicillin resistance was very high at 61%. Metronidazole resistance was detected in 11% of strains: these included 1 Bacteroides fragilis (1%) and 8 Gram positive anaerobes (10%).
Conclusion:
The beta-lactam inhibitor combination drugs (co-amoxiclav and piperacillin-tazobactam), carbapenems, including ertapenem, and metronidazole remain very active against anaerobic organisms and are suitable agents for empiric therapy where anaerobic cover is needed.

MMicPost17 : **False Positive HIV Serological Results of Cadaveric Sera in the Department of Virology at Universitas**

Inéz Rossouw **NHLS/UFS Universitas**
Dominique Goedhals **NHLS/UFS Universitas**

The possibility of false positive HIV results in cadaveric samples was recently discovered in the Department of Virology. HIV test kits are routinely used (worldwide) after death although they are not validated for this purpose. The

phenomenon of a false positive post-mortem HIV test is extremely important in some clinical medico-legal cases when this may be the only test available. Autopsies may be refused based solely on a post-mortem HIV test unnecessarily hampering diagnosis and clarification of causes of death. In some regions in the world tissue transplantation may hinge on a post-mortem HIV test and the unnecesary waste of urgently needed organs may result. The incidence of false positive post-mortem HIV results at the Department of Virology at Universitas is not known and the aim was to determine whether this phenomenon is seen here.

All Rapid HIV tests are confirmed with ELISA tests. Ninety-six cadaveric samples were received from the Department of Anatomical Pathology during the period 2002 to mid-2008. Fifteen were HIV positive, 73 negative and eight indeterminate. An attempt was made to determine the ante-mortem HIV status of the patients with positive post-mortem results by examining medical history and antemortem laboratory tests where available or performing an HIV ELISA test if an ante-mortem bloodsample happened to be stored away.

Of the 15 positive post-mortem HIV results, three were false positive, six confirmed positive and six unknown as too little information was available.

False positive HIV test results, performed on cadaveric samples from the Department of Anatomical Pathology, are seen at the Department of Virology at Universitas. The influence of the time lapse between death and sample harvesting, the harvesting technique and the HIV laboratory test kit used, need to be investigated further. A combination of ELISA tests and molecular techniques should be investigated as method of screening.

MMicPost18 : **An unusual case of Cryptococcus neoformans arthritis in an HIV negative child**

Yesholata Mahabeer NHLS,Nelson R mandela School of Medicine, UKZN
Yacoob Coovadia NHLS, Nelson R mandela School of Medicine, UKZN

INTRODUCTION:
Cryptococcus neoformans is a very uncommon cause of arthritis, found in patients who have a defect in cell-mediated immunity eg corticosteroid use & HIV disease. We describe an unusual case of Cryptococcus neoformans arthritis in a 12 year old HIV negative child.

METHOD

A patient was admitted on 12 July 2009 with spontaneous onset of swelling of his left knee for 2 days and a 1 month history of a swelling on his upper back.
On examination, there was a mass, resembling a cold abscess, on the chest, posteriorly.

There was a fixed flexion deformity of 350. The knee & the chest mass were aspirated & fluid was sent for microbiological culture.

RESULTS
The microscopy revealed many pus cells & a few yeast cells, resembling cryptococci. Cryptococcus neoformans was isolated from both samples.

The patient was commenced on Amphothericin B but by Day 10, it was switched to fluconazole due to renal impairment.

A blood culture, serum cryptococcal antigen test & cerebrospinal fluid sample was sent before amphothericin B was commenced, & these were negative.

A HIV DNA PCR was done on the 30 July 2009 which was negative.

A CT scan of the chest showed destructive lytic lesions of 5th & 7th ribs posteriorly. There was "abscess" formation in relation to the 7th rib.

A CT scan of the knee showed lytic lesions involving the distal medial femoral condyle & articular surface. There was a cortical erosion of lateral tibial metaphysis, in keeping with an infection.

CONCLUSION
We describe an unusual case of C.neoformans arthritis from an HIV negative child. There are fewer than 30 cases of cryptococcal arthritis in the literature & almost all of these were in adults.

MMicPost19 : **The molecular mechanism of ofloxacin resistance in XDR-strains of *Mycobacterium tuberculosis* in Kwazulu-Natal**

Manormoney Pillay	**Department of Microbiology, Nelson R Mandela School of Medicine, UKZN, Durban**
Melendhran Pillay	**Department of Microbiology, NHLS, Inkosi Albert Luthuli Central Hospital, Durban**
Karnishree Govender	**Department of Microbiology, Nelson R Mandela School of Medicine, UKZN, Durban**
A.W Sturm	**Department of Microbiology, Nelson R Mandela School of Medicine, UKZN, Durban**

Background: Mutations in genes encoding GyrA or GyrB subunits have been associated with fluoroquinolone resistance in clinical isolates of M.tuberculosis . Aim(s). To determine the molecular mechanism of ofloxacin resistance in isolates of M.tuberculosis infecting patients in KwaZulu-Natal.

Method(s). Mutations in the gyrA and gyrB genes of the presumed quinolone resistance determining regions of were determined by amplifying and sequencing the

320 and 428 bp products. Minimum inhibitory concentrations (MICs) of ofloxacin were determined using an agar incorporation method.

Results. Four types of gyrase A subunit mutations were observed in ofloxacin resistant isolates of 29 patients: S95 to T; A90 to V; A94 to G and T130 to N. S95 to T was present as a single mutation in 7 isolates of which 5 were XDR and 1 MDR. Two sets of double mutations were observed. One included S95 to T and A90 to V present in 20 isolates all of which were XDR. The second double mutation included S95 to T and A94 to G, present in 1 isolate that was MDR. One XDR isolate contained a triple mutation involving S95 to T, A90 to V and T130 to N.

The F15/LAM4/KZN genotype was identified in 18 (62%) of the 29 isolates. Of these, 17 (94%) were XDR strains.

MICs of 4 and 8 mg per litre were obtained respectively for isolates with the double gyrA mutation S95 to T and A90 to V. The isolate with the double mutation S95 to T and A94 to G showed an MIC of 16 mg per litre. MICs of 1 to 8 mg per litre were observed for the 7 isolates with the single gyrA mutation and 8 mg per litre for the isolate with the triple mutation.

Discussion. Detection of these mutations can be used to predict ofloxacin resistance.

MMicPost20 : **Validating the use of Rifampicin resistance as a surrogate marker for MDR in a high TB prevalence setting in KawZulu-Natal**

Julie Yougabarshanee Moodley	**Department of Microbiology, National Health Laboratory Services, Inkosi Albert Luthuli Hospital**
Melendhran Pillay	**Department of Microbiology, National Health Laboratory Services, Inkosi Albert Luthuli Hospital**
Y.M Coovadia	**Department of Microbiology, National Health Laboratory Services, Inkosi Albert Luthuli Hospital**
L.A Bohlken	**Department of Microbiology, National Health Laboratory Services, Inkosi Albert Luthuli Hospital**

Background: With the advent of HIV, tuberculosis remains a major health issue, with KZN estimated to have the highest rates of TB in South Africa. Dual epidemics of HIV infection and multidrug resistant (MDR) tuberculosis (TB) have severely threatened national and global TB control programmes. Rifampicin resistance is regarded as a surrogate marker for MDR-TB in a TB endemic region.
Objectives. To determine the reliability of Rifampicin resistance as a surrogate marker for MDR-TB in a large TB reference laboratory in KZN
Method(s). This was a retrospective analysis of all positive TB cases with known susceptibility patterns during 9 months from April 2009 to December 2009.
Results.
During the three quarters analysed, the percentage of patients with non-MDR, rifampicin resistant TB ranged from 7.6% to 10.3%

Conclusion. The findings of this preliminary study indicates that in KZN rifampicin resistance is not a reliable surrogate marker for MDR-TB

MMicPost21 *:* Evaluation of rapid serum tests for clinical diagnosis of typhoid fever in an African situation

Arvinda Sooka1, Karen H Keddy1,2, John A. Crump1,2,3,4 Greta Hoyland1Maupi Letsoalo	**1Enteric Diseases Reference Unit, National Institute for Communicable Diseases, Johannesburg, South Africa and 2School of pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa1National Health laboratory Service, Mpumalanga1Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA**

Introduction: Typhoid fever and the emergence of global spread of antibiotic resistant Salmonella enterica serotype Typhi strains are an important public health problem in developing countries.. TUBEX® TF is a rapid diagnostic test that specifically identifies patient IgM against the O antigen of (O9). Typhidot® is a rapid diagnostic test that detects IgG and IgM against a specific 50KD antigen of Salmonella Typhi. Cromotest (Linear Chemicals, S.L.), stained antigens are standardized suspensions of killed bacteria for the detection and semi-quantitation by agglutination in slide or tube tests of human serum.
Objective: Evaluation of four tests: namely TUBEX® TF, Typhidot® and (Widal) (slide and tube) agglutination kits Cromotest were used to test the sensitivity and specificity of tests.
Methodology: Patients were identified from two sites, Mpumalanga province (South Africa) and Tanzania (sub Saharan Africa). Patients who were suspected typhoid fever cases, but had no history of antimicrobial exposure, were included in the study. Blood cultures were taken. Additional blood was obtained from all patients for serological purposes. The following tests were done: TUBEX, Typhidot, Widal slide and tube agglutination.
Results:
A total of 119 patients were enrolled, 80 were South African and 39 were Tanzanian. Ages were available for 49 patients Age range was 0-80 years.
36% ; 16/44 % had blood cultures that were positive for Salmonella Typhi. Blood cultures were negative for 57%; 25/44, the remainder grew other organisms. 12/24 had typhoid fever diagnosed by Widal, 72/72 slide agglutination, 72/72 by TUBEX and 61/72 by Typhidot..
Conclusion:
New serological tests may show promise in rapid diagnosis of typhoid fever. Serology cannot replace current culture methods as additional information such as antimicrobial susceptibilities and molecular epidemiological linkage is lost. Blood culture, prior to initiation of antimicrobial therapy, remains the diagnostic method of choice.

MMicPost22 : New provisional Shigella serotypes in South Africa

Arvinda Sooka1, Nomsa P. Tau1, Anthony M. Smith1,2, Karen H. Keddy1,2, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA)

1Enteric Diseases Reference Unit, National Institute for Communicable Diseases, Johannesburg, South Africa and 2School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Introduction
The genus Shigella is divided into four species: Shigella dysenteriae; serotypes 1-13, S, flexneri; serotypes 1-6, S. boydii serotypes 1-18 and S. sonnei. Since 2003, Enteric Diseases Reference Unit (EDRU) at the National Institute of Communicable Diseases (NICD) has maintained a laboratory-based surveillance system for Shigellosis. Data used during the period of 2003-2010 were used for the investigation of new provisional Shigella serotypes.
Methods
Serological identification of Shigella was performed by slide agglutination with commercially available polyvalent somatic O antigen followed by testing with monovalent antisera. Provisional Shigella serotypes were identified by antisera supplied by Centers for Diseases Control (CDC), Atlanta, United States of America, as part of establishing the unit.
Results
A total of 8560 isolates were received between 2003 and 2010. Of these, 14/8560 (0.2%) cases of laboratory-confirmed provisional Shigella serotypes were characterised over a 7 year period (2003-2010). The provisional serotypes did not agglutinate in established antisera but showed agglutination in the provisional antisera. The majority of isolates were received from Gauteng province (36%; 5/14), followed by Western Cape (29%; 4/14), KwaZulu-Natal (21%; 3/14) and (7%; 1/14) isolate each from Eastern Cape and Mpumalanga. Shigella provisional serotypes were isolated from adults 17-55 years of age and accounted for (9/14; 64%) of all isolates. One isolate was recovered from a blood culture specimen and the remainder from stools. The most common serotype noted was provisional A 3065-93 followed by provisional A 3162-96. No epidemiological linkage was described among these cases. Molecular characterisation will be undertaken to investigate strain relatedness.
Conclusions
Serotyping of Shigella isolates is important for detecting and containing outbreaks. New serotypes may emerge that may guide recommendations for vaccine development and empiric management of disease.

MMicPost23 : Molecular characterization of extended-spectrum β-lactamase producing Shigella isolates from humans in South Africa, 2003 – 2009

Nomsa Tau1, Anthony M Smith1,2, Arvinda Sooka1, Karen H

1Enteric Diseases Reference Unit, National Institute for Communicable Diseases,

Keddy1,2, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA)

Johannesburg, South Africa and 2School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Bacillary dysentery, caused by Shigella species, is an important cause of diarrhoeal diseases. Dysentery caused by Shigella species producing extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase remains a relatively infrequent disease and has been described in countries outside Africa. This study aims to characterize molecular mechanisms of ESBL-production in Shigella isolates in South Africa from 2003 to 2009. All Shigella isolates received by Enteric Diseases Reference Unit (EDRU) were serotyped and antimicrobial minimum inhibitory concentrations were determined using Etests and agar dilution methods. The presence of ESBL-production was established by the disc diffusion screening method. ESBL genotypes (blaTEM, blaSHV and blaCTX-M) and AmpC β-lactamase genotype (blaCMY) were determined using PCR and nucleotide sequencing. Plasmid DNA isolation was conducted to investigate the presence of resistance plasmids. For the years 2003 – 2009, 6833 Shigella isolates were received. Of these, 21 (0.3%) isolates were confirmed ESBL producers; however, only 20 isolates were available for analysis. All ESBL producing Shigella strains showed high levels of resistance to ampicillin, co-trimoxazole, trimethoprim, sulfamethoxaxole, ceftriaxone and cefotaxime. PCR and sequencing results revealed that these isolates carried blaCTX-M-15 (n=18), blaCTX-M-14 (n=1), blaSHV-2 (n=1), blaCMY-2 (n=6) and blaTEM-1 (n=15). Some isolates carried multiple β-lactamase genes including; blaTEM-1, blaSHV-2 and blaCTX-M-15 (n=1), blaTEM-1, blaCTX-M-15 and blaCMY-2 (n=5), blaTEM-1 and blaCTX-M-15 (n=9), blaTEM-1 and blaCMY-2 (n=1). Plasmid DNA was identified in 12 of the ESBL-producing Shigella isolates. Serotype appeared to play no role in production of ESBLs. Although ESBL-producing Shigella isolates in our study comprise less than 1% of all Shigella isolates received for surveillance, they pose a threat to antibiotic therapy. blaCTX-M-15 was the most commonly identified ESBL. This enzyme has been reported to mediate dissemination of ESBL resistance in Shigella through horizontal gene transfer. For this reason continued surveillance is needed to monitor the incidence of ESBL-producing Shigella isolates.

MMicPost24 : **In-vitro activity of tygecycline against clinical isolates of carbapenems resistant isolates of Acinetobacter baumannii in Pretoria**

Nahid H Ahmed University of Pretoria
Kamaldeen Baba NHLS-TAD/University of Pretoria
Ruth Lekalakala NHLS-TAD/University of Pretoria
Anwar A Hoosen NHLS-TAD/University of Pretoria

Objectives: Multi drug resistant Acinetobacter baumannii raises a big therapeutic challenge in our hospital. Tigecycline, a new glycylcyclines with expanded broad

spectrum of activity against multi drug resistant organisms was recently licensed in South Africa. The aim of this study was to evaluate the in-vitro activity of tygecycline against carbapenems resistant Acinetobacter baumannii.

Methods: Consecutive clinical isolates of carbapenems resistant Acinetobacter baumannii were collected between 2009 and 2010 as part of a bigger study. Species identification and susceptibility testing was performed by Vitek-2 system. Modified Hodge test was performed according to the Clinical and Laboratory Standards Institute (CLSI) guideline.

Results: A total of 107 carbapenems resistant clinical isolates of Acinetobacter baumannii were collected. None were susceptible to tazobactam and cefepime; only 2 (2%) were susceptible to ciprofloxacin and 99 (92%) were susceptible to amikacin. A total of 90 (84%) were susceptible to tygecycline, 15 (14%) were intermediate and 2 (2%) were fully resistant.

Conclusion: The use of tygecycline will increase in South Africa in the future and this might result in development of resistance to tygecycline. Therefore continuous monitoring of the susceptibility profile of Acinetobacter baumannii to tigecycline is needed.

MMicPost25 : **Susceptibility Profile of Mycobacterium tuberculosis isolated from patients in Maputo, Mozambique**

Fazana Karim **University of KwaZulu Natal**
Luiza Latifo **University of KwaZulu Natal**
kalaoshnee Ganas **University of KwaZulu Natal**
A Willem Sturm **University of KwaZulu Natal**
Prashini Moodley **University of KwaZulu Natal**

Background: The emergence and spread of multi-drug resistant Mycobacterium tuberculosis (MDR-TB) is a major challenge in many countries. The current 1st line regimen for treatment in Mozambique is rifampicin, isoniazid, pyrazinamide and ethambutol. Surveillance to support this empirical treatment is not routinely performed. The aim of the study was to determine the susceptibility profile of Mycobacteria tuberculosis (MTB) isolated from patients attending 3 primary health care clinics in Maputo.

Methods: Patients were recruited after informed consent was obtained. Sputum specimens were collected and processed for smears and culture at the Central Hospital in Maputo. Isolates were sent to the Medical Microbiology Research laboratories for susceptibility tests using the 1% proportion method.

Results: MTB was isolated from the sputum of 172 of the 349 patients recruited. MDR isolatesd were isolated from 18 patients. One patient harboured an XDR strain.

Conclusion: The prevelance of MDR-TB amongst primary health care clinic attendees was 10%. In 2001, the prevalence of MDR-TB in Mozambique was reported as 3.5% .(Mac-Arthur et al. Int J Tuberc Lung Dis, 2001). There appears to be an increase of MDR TB in Mozambique and this has major public health implications.

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Susceptibility testing of Mycobacterium tuberculosis to Pyrazinamide: Comparison of Nicotinamide activity and the Wayne Method

Reshma Misra *University of KwaZulu Natal*
Prashini Moodley *University of KwaZulu Natal*
A Willem sturm *University of KwaZulu Natal*

Background: Pyrazinamide (PZA), a nicotinamide/niacinamide analog prodrug is an important first line agent for the treatment of infection with Mycobacterium tuberculosis (MTB). The drug is converted into the active bactericidal form pyrazinoic acid by pyrazinamidase (PZase) secreted by MTB. In vitro susceptibility testing for PZA often leads to uninterpretable results due to the challenges of testing for this drug in an acidic medium which has an adverse effect on growth of MTB. Nicotinamide activity is therefore used as a marker of PZA activity. The majority of PZA resistant isolates have mutations in their pncA gene which leads to loss of PZAase activity. The aim of this study was to determine the presence of PZAase activity amongst nicotinamide resistant isolates.
Methods: Stored nicontinamide resistant isolates with varying susceptibility to first and second line anti TB drugs were tested for the presence of PZAase using the Wayne Method.
Results: One hundred and twenty four isolates were tested. Of these, 105 were Wayne test positive whilst nicotinamide resistant.
Conclusion: The majority of the nicotinamide resistant isolates that we tested had normal PZase activity. This may indicate that alternate mechanisms for PZA resistance may be more prevalent in our setting. The genes conferring this resistance have not been elucidated as yet, but most likely lie outside the pncA gene.

MMicPost27 : **Two years experience with MALDI-TOF mass spectrometry in a routine microbiology department of a laboratory in Germany**

Dr Wolfgang Pusch Bruker GmbH Germany
Liezel Raubenheimer Bruker South Africa South Africa

Objectives Introduction of MALDI –TOF mass spectrometry is changing the general workflow in a clinical microbiology laboratory, with the expectation of increased speed and accuracy of microbiological results, thereby enabling improved therapy.

We report about our experiences during two years after introduction of the MALDI Biotyper (MBT) system in our routine laboratory. Methods As a fundamental change, we cancelled all biochemical identifications except for mycoplasma which are not yet in the MBT database. Basic tests such as oxidase, catalase and Staphyloccous coagulase were disposed as not needed. Coagulation tests for subtyping of Shigella, Salmonella, Yersinia, Haemophilus influenzae, pneumococci and meningococci were continued. We also stopped to rely on colour change of chromogenic media, as this is generally only due to one biochemichal reaction and known as not always reliable. Results Introduction of MALDI-TOF MS significantly reduced the usage of consumables in our laboratory. The extensive reference database gives far better results especially for rare microbes, where the biochemical approaches are limited. For instance, Helicobacter pullorum now readily can be distinguished from Campylobacter sp.. Workload has been considerably reduced for Salmonella. The Kligler test prior to sero-subtyping is only needed after an isolate has been identified as Salmonella sp. by the MBT. This frequently eliminates the need for subculture. Identification of anaerobic bacteria is speeded up for several days. Success rate here is at 95%, a 50% increase over our previous biochemichal methods. Yeasts are quickly and reliably identified by the MBT after extracting the cells prior to mass spectrometry with a success rate of > 95%. Conclusion Introduction of MALDI-TOF mass spectrometry for identification of microorganisms has led to considerable positive changes to our lab. It shorted our turn around time, significantly, and improved quality of results. In addition, workload has been reduced and consumption of consumables, too.

MMicPost28 : **Standardised method for fungal identification using liquid culturing and MALDI-TOF mass spectrometry profiling**

Dr Wolfgang Pusch Bruker GmbH
Liezel Raubenheimer Bruker South Africa

Objectives
MALDI-TOF fingerprint analysis became a valuable tool for microorganism identification and classification, recently. While for bacteria and yeasts simple and robust approaches for sample preparation have been reported, the situation for filamentous fungi keeps more complicated. In particular, sample preparation of molds with rigid cell walls is more challenging and the fingerprint spectra derived from spores and mycel show significant differences. Also fingerprint spectra seem to be dependent on cultivation media. We present a novel approach for sample preparation of fungi prior to MALDI-TOF MS improving reproducibility and quality of spectra, significantly.

Methods
Fungal species (e.g. Aspergillus spp., Fusarium spp.) harvested from solid media were grown over night in liquid medium. Subsequently, cell material was harvested by centrifugation, and washed with water. The pellet was dried well and extracted using 35% formic acid/50% acetonitrile. Spectra were analysed using the flexAnalysis software, reference libraries were created and bioinformatic analyses were performed with the MALDI Biotyper 2.0 software (Bruker Daltonik GmbH, Bremen).

Results
Culture in liquid medium has lead to mycelia without spores and thereby largely homogenous cell material. These samples facilitated a successful protein extraction by a simple, short standardised method. Mass spectra generated based on the novel sample preparation method were reproducible and contained many characteristic peaks, thereby leading to a significantly improved identification security. Further, the higher quality of spectra increased the differentiation power of the method and could highlight subspecies differences. A core database of 50 filamentous species has been established and used for first comparative studies. Technical and biological replicates could be identified successfully as well as isolates not included in the database. Reproducibility of results obtained with different mass spectrometers could be demonstrated.

Conclusions
The presented approach may lead to significant improvement of fungal identification in clinical or other routine applications.

MMicPost 29 : Prevalence of bacterial vaginosis and candida species in women receiving termination of pregnancy at Dr George Mukhari Hospital

Caroline maluleka **NHLS-DGMH Microbiology Dept.**
Molebogeng ditsele **NHLS-DGMH Microbiology Dept.**
Marcelle Le Roux **University of Limpopo(Medunsa campus)**
Maphoshane Nchabeleng **NHLS-DGMH Microbiology Dept.**

Background and Aim: Untreated sexually transmitted infections (STI's) have increased the risk of postoperative complications of surgical TOP. Therefore detection and treatment of STI's prior to TOP would improve the outcomes for patients and should be considered as best practice in preserving reproductive health of women. The aim of this study was to determine prevalence of Candida infections and bacterial vaginosis (BV) amongst women presenting for TOP at Dr George Mukhari (DGM) Hospital and to further identify species of Candida that are involved. A study of this nature will provide valuable information leading to the development of intervention strategies for implementation by healthcare administrators.

Methods: Over a 1 month period, 88 women presenting at TOP clinic were enrolled after obtaining an informed consent. A total of 88 high vaginal swab specimens were collected. The swabs were put into saline during collection and in the laboratory, a Gram stained slide was prepared for BV evaluation using the Nugent's score. Swabs were also cultured for 48hrs on Sabaroud's dextrose agar (SDA) medium for yeast isolation. Germ tube was done on specimens which showed growth on SDA medium and those negative were further identified by API - (ID 32C-bioMerieux, Inc).

Results: Of the 88 cultured specimen, 40 (46%) showed growth on SDA. Thirty two (80%) out of the 40 were germ tube positive. The remaining 8 were C sake (2), C

famata (1), Trichospora asahii (1) and 3 could not be identified. Forty one of 88 specimens (47%) were BV positive and 8 had insufficient material. Correlation with clinical presentation is still going to be done

Conclusion: Candida albicans is the commonest species that causes vulvovaginitis in this group of women. BV was also prevalent.

MMicPost30 : **Evaluation of the accuracy and feasibility of implementation of the Genotype MTBDR+ in a routine NHLS TB laboratory in KZN**

Rita Balakistan	**Department of Medical Microbiology, National Health Laboratory Services, Inkosi Albert Luthuli Central Hospital, Durban**
Y.M Coovadia	**Department of Medical Microbiology, National Health Laboratory Services, Inkosi Albert Luthuli Central Hospital, Durban**
Melendhran Pillay	**Department of Medical Microbiology, National Health Laboratory Services, Inkosi Albert Luthuli Central Hospital, Durban**

Background: With the advent of HIV, tuberculosis remains a major health issue, with KZN estimated to have the highest rates of TB in South Africa at 1.7% in 2004. Dual epidemics of HIV infection and multidrug resistant (MDR) tuberculosis (TB) have severely threatened national and global TB control programmes. Since conventional drug susceptibility testing (DST) is time consuming and leads to delays in initiating appropriate treatment, there is an urgent need for the implementation of molecular techniques to rapidly diagnose drug resistant MTB.
Objectives. To report on the performance and feasibility of implementation of the Genotype MTBDR+ assay for the rapid detection of MDR MTB strains during the period January to November 2008.
Method(s). The Genotype MTBDR+ assay was performed on approximately 564 consecutive smear positive sputum samples from patients at increased risk of MDR-TB. Testing was performed in a busy routine NHLS, TB laboratory, KZN, South Africa. Results were compared with the conventional indirect susceptibility testing performed on Middelbrook 7H10 solid agar.
Results. Overall, 97.8% of smear-positive sputum samples were interpretable within 48 hours using the Genotype MTBDR+ assay. Sensitivities of 95%, 92.0% and 91% for MDR, rifampicin mono-resistance and isoniazid mono-resistance respectively were obtained compared with conventional results. Specificities of 99.0%, 97.5% and 98.9.7% for the detection of MDR-TB, rifampicin mono-resistance and isoniazid mono-resistance respectively were obtained compared with conventional results. The overall positive and negative predictive values were found to be 95.8% and 96.8% respectively. The accuracy of the assay was found to be 94.3%
Conclusion. The implementation of the Genotype MTBDR + assay in a busy routine TB laboratory will allow for a more rapid and accurate diagnosis of MDR-TB.

MMicPost31 : **Prevalence of nasal colonization with antimicrobial resistant Staphylococcus aureus amongst medical students at the University of Limpopo, Medunsa Campus.**

Rufus Makwela University of Limpopo(Medunsa)Microbiology Dept
Maphoshane Nchabeleng NHLS-DGMH Micro Dept

BACKGROUND: Methicillin resistant Staphylococcus aureus (MRSA) is one of the common causes of nosocomial infections in many hospitals around the world. Most infections can be difficult to manage resulting in increased morbidity and mortality in both hospitals and in the communities. Health care workers including medical students can be sources of MRSA when they are colonized with MRSA and this is often in the nasal cavities.
In this study we aimed to determine the prevalence of MRSA nasal colonization amongst medical students, comparing the clinical exposed (CE) and the non clinically exposed (NCE) medical students at the University of Limpopo. We also determined the antimicrobial susceptibility patterns of the MRSA isolates to selected antimicrobial agents.
METHODS: A number of 200 nasal swabs from 200 students were collected and processed to isolate MRSA and determine antimicrobial susceptibility profile.
RESULTS: Of 200 students, 80 were NCE and 120 were CE. Out of 200 specimens, 72 methicillin susceptible Staphylococcus aureus (MSSA) and 1 methicillin resistant Staphylococcus aureus were isolated. MRSA isolate had MIC of 1.5 microgram/ml for vancomycin and 0.5 microgram/ml for mupirocin. MSSA showed resistance rate to erythromycin of 28% amongst the CE and 19 % amongst the NCE students.
DISCUSSION: There was only 1 student found to be colonized with MRSA amongst this group of medical students of the University of Limpopo, Medunsa Campus. Colonization with MSSA was 36 %. Other body sites may need to be screened to improve detection of MRSA colonization. However acceptability amongst the students will be a challenge.
CONCLUSION: There was low MRSA colonization in this study. The yield could be improved by sampling more body sites.

83 MMicPost32 : **Prevalence of bacterial vaginosis and candida species in women receiving termination of pregnancy at Dr George Mukhari Hospital**

Caroline Maluleka NHLS-DGM
Mmolebogeng Ditsele NHLS-DGM
Marcelle Le Roux UNIVERSITY OF LIMPOPO(MEDUNSA)
Maphoshane Nchabeleng NHLS-DGM

Department of Medical Microbiology. University of Limpopo, Medunsa Campus.

Background and Aim: Untreated sexually transmitted infections (STI's) have increased the risk of postoperative complications of surgical TOP. Therefore detection and treatment of STI's prior to TOP would improve the outcomes for patients and should be considered as best practice in preserving reproductive health of women. The aim of this study was to determine prevalence of Candida infections and bacterial vaginosis (BV) amongst women presenting for TOP at Dr George Mukhari (DGM) Hospital and to further identify Candida species that are involved. A study of this nature will provide valuable information leading to the development of intervention strategies for implementation by healthcare administrators.

Methods: Over a 1 month period, 88 women presenting at TOP clinic were enrolled after obtaining an informed consent. A total of 88 high vaginal swab specimens were collected. The swabs were put into saline during collection and in the laboratory, a Gram stained slide was prepared for BV evaluation using the Nugent's score. Swabs were also cultured for 48hrs on Sabaroud's dextrose agar (SDA) medium for yeast isolation. Germ tube was done on specimens which showed growth on SDA medium and those negative were further identified by API - (ID 32C-bioMerieux, Inc).

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Conclusion: Candida albicans is the commonest species that causes vulvovaginitis in this group of women. BV was also prevalent.

MMicPost33 : Comparison of an automated system with conventional methods, for the identification and / or antifungal susceptibility testing of Candida spp

Dianithi Naidu	**NHLS , Department of Medical Microbiology, IALCH , Durban.**
Prof Y.M.Coovadia	**NHLS , Department of Medical Microbiology, IALCH , Durban**

INTRODUCTION :
Invasive candidiasis remains an important cause of morbidity and mortality in immunocompromised and intensive care unit patients. With the emergence of antifungal resistance , it is important for laboratories to speciate the candida isolates as well as determine their susceptibilities.

AIM :
To compare the Vitek 2 System (BioMèrieux) for the identification and /or antifungal susceptibility of Candida spp with the API ID32 C (BioMèrieux) and the E- Test(AB BIODISK).

METHODS :

Fifty Candida species, isolated from clinical specimens were identified by the Vitek 2 and the API ID 32 C systems.
Antifungal susceptibilities to amphotericin B, fluconazole and voriconazole were determined using the Vitek 2 AST – YS01 card and the E-test , according to the manufacturer's instructions.
RESULTS :
Forty eight of the 50 Candida isolates were correctly identified by both the Vitek2 system and the API ID 32 C .Two isolates identified as Candida sake by the API system were reported as C. parapsilosis by the Vitek 2 system. The morphology was consistent with that of Candida sake.

All candida isolates were found to be susceptible to fluconazole by both methods. Two isolates tested by the Vitek 2 system showed discrepant results when compared to the E- test method. Candida glabrata was reported as resistant to voriconazole and Candida parapsilosis as resistant to amphotericin by the Vitek 2 system.These isolates were susceptible by the E- test method.

CONCLUSION :
The Vitek-2 system compared well with conventional methods for the identification and antifungal susceptibilities of Candida species. The advantage of this automated system is that it provided an identification and antifungal susceptibility result within 18 hours as compared to the E- test and API ID 32 C which took 24 to 48 hours.

MMicPost34 : **Evaluation of rapid serum tests for clinical diagnosis of typhoid fever in an African situation**

Arvinda Sooka[1], Karen H Keddy[1,2], Maupi Letsoalo[3], Greta Hoyland[4], Claire Lise Chaignat[5], Anne B. Morrissey[6], John A. Crump[6,7,8,9]

[1]Enteric Diseases Reference Unit, National Institute for Communicable Diseases, Johannesburg, South Africa
[2]Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[4]National Health Laboratory Service, Nelspruit, South Africa
[3]Statistical Support Services, Directorate: Research and Innovation, Tshwane University of Technology, Pretoria, South Africa
[5]World Health Organisation, Geneva Switzerland
[6]Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA
[7]Duke Global Health Institute, Duke University, Durham, North Carolina, USA
[8]Kilimanjaro Christian Medical Centre (KCMC), Moshi, Tanzania,
[9]Kilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania

Introduction:
Typhoid fever and the emergence of antibiotic resistant *Salmonella enterica* serotype Typhi strains are an important public health problem in developing countries. Rapid

diagnostics tests may facilitate diagnosis in developing countries. TUBEX® TF is a rapid diagnostic test that specifically identifies patient IgM against the O antigen of (O9). Typhidot® is a rapid diagnostic test that detects IgG and IgM against a specific 50KD antigen of *Salmonella* Typhi. Widal test is a standardized suspension of killed bacteria for the detection and semi-quantitation by tube agglutination tests.

Objective:
Widal agglutination and semi-quantitative slide (Cromotest®, Linear Chemicals, Spain) TUBEX® TF and Typhidot® were evaluated against blood culture (BC) as the gold standard.

Methodology:
Patients were identified from two sites, Mpumalanga Province (MP), South Africa and Moshi, Tanzania. Blood cultures were taken from suspected typhoid fever cases from Mbombela district, MP between 2006 and 2009. Tanzanian blood samples were collected as part of a surveillance programme for aetiology of bacteraemia presenting to KCMC. Additional blood was obtained from all patients for serological purposes.

Results:
Ninety-two patients were enrolled: 53 from South Africa and 39 from Tanzania. Age range was <1 year to 80 years. *Salmonella* Typhi was cultured from 28/92 (30%) of blood cultures. Other pathogens were cultured from 12/92 (13%) and 52/92 (57%) of blood cultures were negative. Kappa statistics of -0.017 (95% CI -0.130 - -0.097) and -0.070 (95%CI -0.221 – 0.080) indicates that there is no agreement and fair agreement between semi-quantitative O slide agglutination and Widal O-titres respectively and BC. Kappa statistics of 0.309 (95% CI 0.098 – 0.520) and 0.112 (95%CI -0.056 – 0.281) indicates that there is no agreement and fair agreement between semi-quantitative H slide agglutination and Widal H-titres respectively and BC. Kappa statistics of 0.391 (95% CI 0.199 – 0.582) indicates that there is slight agreement between TUBEX® TF and BC and kappa statistics of 0.351 (95%CI 0.139 – 0.563 and 0.371 (95% CI 0.166 – 0.573) indicates there is slight agreement between Typhidot® IgM and IgG respectively and BC.

Conclusion:
Older and new serological tests may assist in rapid diagnosis of typhoid fever, but do not correlate with blood culture. Semi-quantitative slide agglutination may be unreliable. New serological tests may assist in rapid diagnosis of typhoid fever, but cannot replace current culture. Antimicrobial susceptibilities and molecular epidemiological linkage cannot be elicited on serological diagnosis. Blood culture, prior to initiation of antimicrobial therapy, remains the diagnostic method of choice.