1. The use of Microsens TB-Beads as a means of concentrating Mycobacterium tuberculosis from sputum

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Background: Concentrating and lysing Mycobacterium tuberculosis bacilli in sputum are probably the most critical steps in ensuring sensitivity of downstream molecular detection assays. Classically this involves centrifugation which is expensive, labour intensive and unsuitable for point of care testing. TB-Beads (Microsens) which utilize magnetic bead technology to bind M. tuberculosis from sputa have been used to concentrate bacilli for smear microscopy. We aimed to determine whether this technology could replace centrifugation as a concentration step prior to molecular detection of TB.

Method: Initial experiments involving the comparison of 14 mucolytic agents showed dithiothreitol (DTT) in NaOH to be a potent mucolytic. GeneXpert®-negative sputa were pooled, homogenised and stored in 1 ml aliquots. Sputa were spiked with 1x10⁶ CFU of M.tuberculosis H37Rv. Spiked and unspiked sputa were liquefied with either NALC-NaOH or DTT-NaOH. For the conventional method sputa were centrifuged at 3000 rpm for 15 minutes and the pellets resuspended. The Microsens TB-Bead method was performed according to the manufacturer’s instructions. A modified TB-Bead method was also included. In-house lysis buffer was added to the pellet or eluted bacilli. Specimens were sonicated to lyse the concentrated bacteria, heat-killed and subjected to real-time PCR for M.tuberculosis to compare the efficiency of the concentration methods.

Results: The conventional method of concentrating bacteria was marginally better but similar to the Microsens TB-Bead method prior to molecular detection. An improvement was also noted with the use of DTT-NaOH as compared to NALC-NaOH. The modified TB-Bead method also yielded better results compared to the Microsens TB-Bead protocol.

Conclusion: The use of Microsens TB-Beads in combination with the DTT-NaOH mucolytic agent is a promising method for concentrating M.tuberculosis bacilli prior to molecular detection, and may be more suitable for point-of-care testing than conventional concentration techniques.

2. Prevalence and characterisation of hepatitis B infection in HIV-infected pregnant women at Tygerberg Hospital, Cape Town, South Africa

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Background: Immunisation protocols in many African countries are predicated on the belief that hepatitis B virus (HBV) transmission occurs predominantly horizontally after birth rather than during birth. For late immunisation to be effective HBV-infected mothers must be of “low infectivity”. Little is known about chronic HBV infection in human immunodeficiency virus (HIV)-infected pregnant women where HBV replication may be increased. The aim of this study was to determine the prevalence and character of HBV infection in HIV-infected pregnant women.

Method: We obtained ethical approval to conduct a retrospective study using all available stored plasma samples from HIV-infected pregnant women who delivered at Tygerberg Hospital between July 2008 and October 2009. Samples were anonymised and tested for HBsAg and anti-HBc. Those confirmed HBsAg-positive by neutralisation were tested for HBeAg and anti-HBe (AxSYM®, Abbott, Chicago, IL) and had HBV viral load testing and genotyping performed.

Results: Of 1661 HIV-infected women, 202 had samples available. The median age was 28 years (interquartile range 24-32), the median CD4 count 198×10⁶/l (interquartile range 117-329) and 96/202 (47.5%) were on antiretroviral therapy. The overall prevalence of plasma HBsAg was 5.9% (12/202). Four (33.3%) of 12 were HBeAg positive. Four of 10 HBsAg-positive women with available sample had HBV viral loads >10⁶ copies/ml. Sequencing data revealed that nine samples were genotype A and one genotype D. No surface or polymerase mutations were found. Serum anti-HBc in the absence of detectable HBsAg was detected in 75/178 women indicating past infection in 42%.

Conclusion: There is a high prevalence of HBeAg positivity and high HBV viral loads in this cohort. The results suggest HIV and HBV co-infected women may be at an increased risk of transmitting HBV to their babies. Further studies are necessary to determine if there is justification for bringing HBV immunisation closer to the time of birth.
3. *Mycobacterium tuberculosis* isolates cultured in the presence and absence of oxygen induces cytotoxicity in A549 alveolar epithelial cells

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**Background:** The emergence of drug-resistant (DR) *Mycobacterium tuberculosis* and our previous findings that clinical isolates of DR *M. tuberculosis* adhered to and invaded the alveolar epithelial cells more effectively than the virulent laboratory strain H37Rv highlight the need to include isolates grown under oxygen deprivation in research on this organism. In this study, we report on *M. tuberculosis*-induced cytotoxicity in A549 alveolar epithelial cells in vitro.

**Method:** Fifteen clinical isolates (Beijing and F15/LAM4/KZN families and non-clustering strains), and two laboratory strains of *M. tuberculosis* were used to infect A549 cells. All isolates were grown in the presence of oxygen and under oxygen deprivation. We quantified the amount of lactate dehydrogenase (LDH) released from A549 cells after infection to evaluate necrosis using the Cyto Tox 96® kit (Promega).

**Results:** The isolates grown under oxygen deprivation had a higher level of cytotoxicity than those grown in the presence of oxygen. Cytotoxicity levels induced by F15/LAM4/KZN and Beijing isolates grown under oxygen deprivation ranged from 18.4%-35.7% and 17.7%-27.4% respectively. The highest level (35.7%) was produced by an extensively drug resistant (XDR) strain of the former. Growth in the presence of oxygen resulted in lower cytotoxicity, ranging from 14.3%-22.4% by the F15/LAM4/KZN strains, and 13%-23.3% by the Beijing strains. Non-clustering strains induced between 3.5%-8.5% (oxygen) and 5.3%-10.7% (oxygen-deprived). The virulent and avirulent laboratory strains induced cytotoxicity levels of 3.9% and 2.9% (oxygen) respectively and 5.7% and 3.2% (oxygen-deprived) respectively.

**Conclusion:** These results correlate well with our previous findings on the adhesion and invasion rates of these isolates. The increased tissue destruction induced by the F15/LAM4/KZN (XDR) strain compared to the others attest to its virulence and may partly explain the high mortality rates of patients infected with this strain in KwaZulu-Natal in 2005.

4. *Treponema pallidum*: macrolide resistance and molecular subtyping of strains from South Africa

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**Background:** *Treponema pallidum*, the causative agent of syphilis, cannot be cultivated. As standard antimicrobial testing is impossible, other methods are required to test for antimicrobial resistance. The aim of this study was to determine if the 23S rRNA point mutation (A2058G) that confers macrolide resistance was present in *T. pallidum* identified in genital ulcer specimens in South Africa and to assess the level of heterogeneity among *T. pallidum* strains causing ulcers.

**Method:** The study group included participants of both the Sexually Transmitted Infections National Microbiological Surveillance programme and an acyclovir episodic therapy study recruited in Gauteng. Extracted DNA was screened for *T. pallidum* using a real-time multiplex PCR assay. Sixty positive samples were confirmed with a commercial *T. pallidum* real-time PCR assay. These *T. pallidum*-positive DNA extracts were screened for the A2058G point mutation in the peptidyltransferase region of the 23S rRNA subunit using a rapid PCR-based restriction digest assay. Syphilis subtyping was done on all positive samples, based on two variable treponemal genes (arp and tpr).

**Results:** No point mutations were present among the 60 *T. pallidum* DNA samples that could imply macrolide resistance. A total of eight arp repeat sizes, eight restriction fragment length polymorphism patterns and a combined total of 17 subtypes were identified in this study population. The most common subtypes were 14d (43%), followed by 17d (13%), 14b (7%), 17b (5%), 22b (5%) and 23b (5%).

**Conclusion:** This was the first study in South Africa to examine both macrolide resistance profiles and subtype distribution of *T. pallidum* strains. Macrolide-resistance in *T. pallidum* is unreported in Africa but could emerge through drug pressure or importation in the future. The arp and tpr gene subtyping is a useful tool to study epidemiologically related strains and surveillance should be implemented in bigger sample size and over longer time to determine trends.

5. Emerging penem resistance in *Salmonella* species: what option left for the therapy of ESBLs?

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**Background:** Broad-spectrum carbapenem group is the current therapy for strains of *Enterobacteriaceae* that express extended spectrum β-lactamases (ESBLs). However, recent reports of therapeutic failures of penems with strains that produce multiple β-lactamases are being documented. This study profiled antibiotic resistance in clinical isolates of *Salmonella* species in a tertiary hospital in the Eastern Cape of South Africa with the aim of identifying the current state, success or failure of *Salmonella* therapy in the region.

**Method:** One hundred and twenty clinical isolates of *Salmonella* were screened. The identity and antibiogram of each isolate was performed with a modified Kirby–Bauer method. Antimicrobial susceptibility testing was performed by the disk-diffusion method. The antibiotic resistance patterns were adjudged, ranking order of importance as percentage of each type of resistance for each sample evaluated. Molecular characterisation of β-lactamase genes was carried out by examining isolates for the presence of ESBL enzyme blaTEM, blaTEM*, blaSHV, blaCMY-2 and blaCTX-M types.
Results and conclusion: A considerable portion of the isolates (58/120) showed multivalent resistance to commonly used drugs and even more to the first-line antibiotics, including ampicillin and amoxicillin. Of the 58 multiple-resistant isolates, 14 (24.1%) were resistant to one or more of the penems examined. One strain was particularly resistant to all the three penem drugs and all the other antibiotics except cefepime. Of the 120 Salmonella isolates, 61 (50.7%) were ESBL positive by genotypic determination. A total of nine (14.7%) and 54 (88.5%) of the 61 isolates were CMY-2 and TEM β-lactamase producers respectively. Only one of the Salmonella spp. was CTX-M ESBL producer. The emerging resistance of Salmonella isolates to carbapenem drugs is spelling a doom in therapy, leaving fewer or no options in the choice of drugs for ESBLs. Further studies are required to identify the genes possibly coding for penem resistance in these strains.

6. Evaluation of the EntericBio® Multiplex PCR system for the detection of bacterial enteric pathogens in an academic hospital setting

Background: Conventional culture has been regarded as the “gold standard” method for the detection of bacterial enteric pathogens with the disadvantage of several days turnaround time and the occurrence of nonculturable isolates. The EntericBio® multiplex PCR system simultaneously detects Campylobacter spp., Salmonella enterica, Shigella spp. and Escherichia coli 0157 from faecal samples through an overnight broth enrichment followed by PCR amplification and detection by hybridisation.

Method: In this study of 100 samples, including clinical specimens and stool samples spiked with ATCC control strains, a comparison of the multiplex PCR system and routine culture was done.

Results: The EntericBio® system yielded fewer negative results (83%) in comparison to the 88% negative results obtained through culture. Overall, sixty-six (66%) of the PCR-analysed samples gave results concordant with that found with routine culture. Of the analysed clinical specimens, discordant results were obtained for 6/59 samples. Of these, five specimens had organisms detected by PCR but not by routine culture (Shigella spp. n=3, Campylobacter spp. n=3, S. enterica n=1). In one culture Salmonella group B was detected, but this could not be confirmed by PCR. Conventional culture taken as the gold standard, the EntericBio® multiplex PCR system showed a sensitivity, specificity and negative predictive value of 80%, 92% and 98% respectively.

Conclusion: In the evaluated laboratory setup, the EntericBio® system proved to be convenient to use with a turnaround time superior to that of conventional culture while showing a high negative predictive value which would make it a useful first-line screening method for bacterial enteric pathogens. It further has the potential to improve the detection of bacterial enteric pathogens such as Campylobacter spp. which can be difficult to culture.

7. Shunt-related infections: meningitis in neurosurgery patients in a large referral hospital in KwaZulu-Natal, South Africa

Background: Shunt infection is a life-threatening complication of shunt surgery. When a shunt infection is encountered, antibiotic treatment must be administered without delay, necessitating the use of empirical treatment while specimens are being processed in the laboratory. Effective empirical treatment depends on knowledge of the spectrum of organisms causing shunt infections and their susceptibility patterns. At present, this information is not available for the neurosurgery wards at the Inkosi Albert Luthuli Central Hospital, Durban. In this study we seek to address this issue by documenting the organisms which were implicated in shunt infections during 2009 and 2010, and their antimicrobial susceptibility patterns. To our knowledge, a data analysis of this kind has not previously been undertaken in KwaZulu-Natal.

Method: Data extracted from the hospital microbiology database will be analysed to reveal the organisms causing cerebrospinal fluid shunt infections, and their antibiotic susceptibility patterns. Differences between age-groups and sexes will also be examined.

Results: Provisional findings show that coagulase-negative staphylococci were the commonest organisms (35%), followed by acinetobacters (27), Klebsiella pneumoniae (15), Staphylococcus aureus (14), other Gram-negative bacilli (27), other Gram-positive cocci (22), Gram-positive bacilli (2) and yeasts (7). Seventy-two per cent of coagulase-negative staphylococci and 75% of S. aureus were resistant to clindamycin. All staphylococci were susceptible to vancomycin. Of the acinetobacters, 98% were resistant to cefotaxime, 64% resistant to carbapenems, 23% resistant to colistin but 81% were susceptible to amikacin. Ninety per cent of Klebsiella pneumoniae were ESBL positive, and 28% resistant to ciprofloxacin; all were susceptible to carbapenems and colistin.

Conclusion: Over 72% of staphylococci were resistant to clindamycin, but remained susceptible to vancomycin. 90% of K. pneumoniae were extended spectrum β-lactamase-positive but fully susceptible to carbapenems. Acinetobacters displayed high levels of resistance to most antibiotics tested; however, over 77% were susceptible to amikacin and colistin.

8. Clinical and diagnostic findings in children involved in an enterovirus meningitis outbreak in the west of Pretoria, South Africa

Background: An outbreak of enterovirus meningitis occurred in the west of Pretoria between October 2010 and
February 2011. We reviewed the clinical and diagnostic characteristics of children involved in the outbreak.

Method: A retrospective review was carried out of children with positive enterovirus PCR on cerebrospinal fluid (CSF) specimens. The patients were admitted to the paediatric service of a large regional hospital.

Results: CSF specimens from 26 patients presenting to Kalafong hospital with symptoms and signs suggestive of meningitis had positive PCR results for enterovirus. Twelve of the cases were male (46%) with a median age of 5 years and 5 months (range 6 weeks to 10 years). The dominant clinical symptoms were fever (88%), headache (76%) and vomiting (72%). In 64% of the patients signs of meningeal irritation were recorded. Convulsions occurred in two of the patients. CSF microscopy revealed pleocytosis in 22 (88%) of cases with neutrophil predominance in 14 (56%). Of the 15 patients with known human immunodeficiency virus (HIV) status, only one was HIV-positive. Her presentation differed from the rest as she had a very high C-reactive protein (CRP, 390mg/l). No other cause for the raised CRP was found. The median duration of hospital admission in the review was five days (1-8 days). In 25 of the patients empiric therapy with a third-generation cephalosporin was started. This was discontinued early when the enterovirus PCR came back positive. An analysis of CSF samples reported by Wolfardt et al. revealed echovirus 4 as the main serotype associated with this outbreak.

Conclusion: There are many diseases with similar CSF findings as those found in viral meningoencephalitis. In this group of patients early definitive diagnosis could reduce the length of hospital stay as well as the empirical use of parenteral antibiotics. This has both financial and morbidity implications.

9. Hepatitis B serological markers and vaccination coverage in South African healthcare workers

Method: A cross-sectional sero-survey of healthcare workers (HCWs) and their patients was conducted in the healthcare setting, thus care health workers (HCWs) and their patients are at risk for acquiring HBV infections. Studies on HBV seroprotection and vaccination status of HCWs are limited in South Africa. This study was conducted on Gauteng HCWs during 2009-2010 in order to (1) investigate HBV serological markers and (2) investigate associations between HBV vaccination and HBV serological status, and demographics.

Method: This was a cross-sectional sero-survey of Gauteng HCWs (42 final-year nursing students from three colleges, 44 nurses working in private and public hospitals in Tshwane, and 27 HCWs working in the private and public sectors of Ekurhuleni). Blood samples were drawn following informed consent, and tested for HBsAg, anti-HBc, and anti-HBs, using the Elecsys® 2010 Immunooassay System (Roche Diagnostics, Penzburg, Germany). Data on age, gender, years as a HCW and number of HBV vaccination doses received were also collected by means of a questionnaire. Data was captured and analysed using Microsoft® Access (Microsoft Office 2007) and further analysed using Epi Info® version 3.5 (Centers for Disease Control and Prevention, 2008).

Results: The majority of participants, 78/113 (69%) had received at least one dose of vaccine but only 28.3% (32/113) were fully vaccinated. Four (3.5%) were HBsAg positive, and 23.9% (27/113) had been exposed to natural infection, with 18.6% (21/113) being protected through natural infection. Anti-HBs alone (indicating vaccination) was found in 52.2% (59/113), with protective levels in 46.9% (53/113).

Conclusion: Vaccination uptake and the seroprotection rate are suboptimal among HCWs in Gauteng Province, resulting in almost a quarter of them being at high risk for acquiring HBV infection, and 3.5% being at risk of transmitting HBV to their patients.

10. Analysis of common microorganisms isolated at the haematology unit of a central hospital in Durban, KwaZulu-Natal, South Africa

Method: Data collected included demographics, information regarding the microbiological laboratory, type of specimen, name of isolate and antibiogram from the Haematology Unit. This was a prospective study of all bloodstream infections. The audit revealed that on average 47% of all bloodstream infections were caused by Gram-positive bacteria, 22% by Gram-negative bacteria and 5% by fungi. The audit revealed that on occasion only two nurses were allocated to care for 18 patients.

Conclusion: There was no nosocomially acquired microorganism-related outbreak of infection at the Haematology Unit in 2010. Isolation precautions and strict infection control measures are advised. The patient-to-nurse ratio is unacceptably high for the nursing of neutropenic patients.
11. The prevalence of cryptococcal meningitis in patients admitted in medical wards at Dr George Mukhari Hospital

Chephe TJR, Makhado NA, Maloba B, Nchabeleng M

Method: A retrospective record of results analysed from January 2008 to December 2010 were retrieved from the Laboratory Information System and analysed. Diagnosis was done by staining cerebrospinal fluid with India ink, Gram’s staining, antigen detection, or by culture.

Results: Of the 2021 CSF samples tested, 209 (10.34%) gave significant growth. Of the 209 significant isolates, 189 (90.43%) were Cryptococcus neoformans. The total number of patients screened was 690, 593, and 738 for the three consecutive years. The percentage positive for all pathogens was 10.00%, 9.61% and 11.25%, respectively. The positive rate contributed by Cryptococcus neoformans was 9.28%, 8.60%, and 10.03% which calculates to 92.75%, 89.47% and 89.16% of the total isolates.

Conclusion: Cryptococcus neoformans is the single most important cause of meningitis in hospitalized patients at Dr George Mukhari hospital. Although the trend seemed to come down in 2009, a sharp ascend occurred in 2010.

12. The prevalence of cryptococcal meningitis in HIV-positive patients at Dr George Mukhari Hospital, South Africa

Chephe TJR, Makhado NA, Maloba B, Nchabeleng M

Method: A retrospective record of cerebrospinal fluid (CSF) samples tested from January 2008 to December 2010 were retrieved from the Laboratory Information System and analysed. Diagnosis was done by staining cerebrospinal fluid with India ink, Gram’s staining, antigen detection, or by culture.

Results: CSF samples from 747 HIV positive patients were tested. Of the 747 HIV positive patients, the total number of patients screened was 218, 229, and 300 for the three consecutive years. Cryptococcus neoformans was isolated in 19 (8.72%), 21 (9.17%), and 34 (11.33%) CSF samples during 2008 to 2010, respectively.

Conclusion: Despite significant advances in the treatment of HIV infection with ARV rollout across the country, there is still evidence that prevalence of cryptococcal infection is still in patients admitted at Dr George Mukhari hospital.

13. Prospective observational study to assess hand skin condition after application of alcohol-based hand-rub solutions

Deleplanque T

Method: 231 HCWs were included, 33.8% nurses, 22.1% nurse assistants and 14.7% hospital cleaners. (Mean age 40 years). A self-assessment questionnaire was administered in order to collect information about HCWs, their practice of hand hygiene and tolerance of ABHRS (Aniosgel NPC 85® Anios, Lille, France) during daily use.

Results: The skin hydration increased significantly after application of ABHRS for the two sites of measurements (P = 0.0001). The mean of pH values did not change significantly on the back of hand, but there were a significant changes for the palm (-0.069 ± 0.41, P = 0.012). Superficial sebum content decreased significantly after rub on the palm (-0.53 ± 1.56, P = 0.0001), but no significant difference was observed for the back (P = 0.076).

Conclusion: ABHRS are well tolerated and do not dry the skin; pH and superficial sebum values decreased slightly, but not affected the skin barrier function. Values remained within the physiological range. This study provides a strong argument to encourage HCWs to intensive use of ABHRS, in order to improve hand hygiene compliance and reduction of nosocomial infections.

14. A case of fatal pneumonia and spontaneous bacterial peritonitis caused by Bordetella bronchiseptica in a patient with alcoholic liver disease

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Method: A diagnosis was done by staining cerebrospinal fluid with India ink, Gram’s staining, antigen detection, or by culture.

Results: CSF samples from 747 HIV positive patients were tested. Of the 747 HIV positive patients, the total number of patients screened was 218, 229, and 300 for the three consecutive years. Cryptococcus neoformans was isolated in 19 (8.72%), 21 (9.17%), and 34 (11.33%) CSF samples during 2008 to 2010, respectively.

Conclusion: Despite significant advances in the treatment of HIV infection with ARV rollout across the country, there is still evidence that prevalence of cryptococcal infection is still in patients admitted at Dr George Mukhari hospital.

Background: Bordetella bronchiseptica is a pleomorphic gram-negative coccobacillus that is found as a commensal in the upper respiratory tracts of wild and domestic animals where it may cause infection. Humans can become colonized in the respiratory tract, which makes interpretation of results difficult if the organism is isolated from the respiratory tract secretions. Human infections rarely occur, the most common
being respiratory tract infections. A history of contact with animals is helpful, but if negative it does not exclude *B. bronchiseptica* infection. Human infections are usually associated with immunosuppression, particularly acquired immunodeficiency syndrome (AIDS).

**Case report:** A 42 year old female presented with a three week history of abdominal distention and vomiting. Other complaints were loss of weight, loss of appetite and painful feet. There was a strong history of alcohol abuse and smoking. Further inquiry revealed no history of animal exposure. Clinical examination revealed signs of alcohol liver disease and a massive ascites. There were no abnormal respiratory findings but a chest x-ray done on admission revealed opacification of the right upper lobe of the lung. A subsequent chest X-ray done 3 days later showed progression of opacity with involvement of almost the entire right lung. * Bordetella bronchiseptica was isolated from the sputum and ascitic fluid specimens. The patient demised 3 days later despite treatment with ciprofloxacin.

**Discussion:** This case illustrates the potentially aggressive nature of this organism in susceptible patients. Immunosuppression due to alcoholic liver disease was the most likely predisposing factor for the infection in this patient. Although peritonitis in patients on continuous ambulatory peritoneal dialysis has been previously reported in the literature, to our knowledge, this is the first case of spontaneous bacterial peritonitis caused by *B. bronchiseptica."

15. Sequence analysis of known resistance conferring genes to fluoroquinolones and aminoglycosides among *Mycobacterium tuberculosis* isolates from KwaZulu-Natal, South Africa

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**Background:** South Africa is one of the high burden countries of *Mycobacterium tuberculosis* (TB) infection. Despite multi-drug regimens for the treatment of TB, the emergence of drug resistant strains of *M. tuberculosis* has severely compromised TB therapy. The fluoroquinolones and aminoglycosides are the most effective second line anti-tuberculosis drugs. In this study we evaluated a possible correlation between MICs and mutations in genes known to confer resistance to these agents.

**Method:** The isolates for this study were chosen on the basis of susceptibilities to each of the drugs. MICs were determined by incorporating varying concentrations of fluoroquinolones (ciprofloxacin, ofloxacin and moxifloxacin) and the aminoglycosides (kanamycin, amikacin and capreomycin) into Middelbrook 7H11 media. Regions of interest of the *gyrA*, *gyrB* genes (fluoroquinolone resistance) and the *rrs* gene (aminoglycoside resistance) were amplified by PCR and sequenced. Single nucleotide polymorphisms were identified in the final nucleotide sequences in comparison to the H37Rv reference strain.

**Results:** Sequencing revealed 2 polymorphisms in the *gyrA* gene (G284C, C269T) but no polymorphisms in the *gyrB* gene. The G284C polymorphism was present in 21/22 fluoroquinolone resistant isolates in 11/13 drug susceptible isolates with varying MIC profiles. The C269T polymorphism was observed in 16/22 fluoroquinolone resistant isolates and was absent in all susceptible isolates. Additionally, 6 fluoroquinolone resistant isolates showed only the G284C polymorphism present in the *gyrA* and no polymorphisms in the *gyrB* genes despite showing high levels of resistance to fluoroquinolones. Screening of the *rrs* revealed only the A1401G polymorphism, detected in all 22 of the aminoglycoside resistant isolates each with an MIC profile reflective of high levels of resistance to aminoglycosides.

**Conclusion:** Detection of the A1401G polymorphism correlates with aminoglycoside resistance whilst detected mutations in the *gyrA* gene do not correlate with phenotypic resistance to fluoroquinolones.

16. An evaluation of two microscopy techniques for the detection of *Cryptococcus neoformans* in cerebrospinal fluid

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**Background:** In South Africa there is a significantly high burden of cryptococcosis among HIV infected patients. The increased mortality in HIV-Cryptococcal co-infections, reinforces the need for simple, inexpensive, rapid and accurate methods for the laboratory diagnosis of cryptococcal meningitis.

**Method:** A comparative evaluation of two staining methods was performed on 90 CSF samples from patients with suspected Cryptococcal meningitis using culture as the gold standard. Gram and India ink staining was performed according to standard laboratory procedures.

**Results:** 83 of 90 specimens were culture positive for *Cryptococcus neoformans*. Sensitivities of 84% and 89% were obtained for the India ink and Gram stain respectively. Specificities were found to be 100% for both microscopy techniques.

**Conclusion:** The Gram stain, which is routinely performed on all CSF specimens, was found to be more sensitive and easier to read than India ink.

17. Candida species isolated from the oral mucosa from a South African population of HIV-positive women

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**Background:** *Candida* infections are known contributors to the higher morbidity and mortality rates seen in HIV-positive patients, especially in underdeveloped countries. Females are more predisposed to *Candida* infections than their male counterparts. The objective of this study was to investigate the prevalence of *Candida* species in HIV-infected South African females and to compare their colonization and susceptibility patterns with other parameters that may be distinguishing in women.

**Method:** Samples were collected from 146 HIV-positive...
women with either a white pseudomembranous plaque on the tongue or visible oral candidiasis. Sample collection was done in community hospitals in the Western Cape, South Africa. Data from the patient’s hospital folder was also collected. Positive Candida samples were differentiated by growth in selective and chromogenic media and fluconazole drug resistance was investigated using the disk diffusion method in Yeast Nitrogen Base agar.

**Results:** *Candida albicans* was the species most isolated from late stage immunosuppressed patients. A marked increase in *C. albicans, C. dubliniensis* and *C. glabrata* species in the 21-50-year-old groups was seen, with most of it occurring in the 31-40 age group. A significant association between *Candida* species colonization and health status was found, as well as between *Candida* species and drug susceptibility results.

**Conclusion:** This study emphasizes the need for ARV compliance and monitoring of these patients, as the numbers of invasive *Candida* species seemed to decrease after continuous anti-retroviral treatment.

The relatively low levels of fluconazole resistance seen in *C. albicans* and susceptibility of all *C. dubliniensis* isolates are encouraging, and could be related to the improved ARV treatment and compliance tracking these patients received in public hospitals. *C. albicans* was the only species isolated from pregnant/recently pregnant women. Because an association between pregnancy outcomes and *Candida* has previously been reported, this deserves further investigation.

18. Impact of rotaviral gastroenteritis outbreaks on nosocomial sepsis rates in a neonatal unit

**Background:** Rotavirus infection (RVI) is a well-recognised cause of nosocomial gastroenteritis in neonatal units (NNU) worldwide. Concomitant bacterial sepsis may occur, however there are no published reports of increased nosocomial bloodstream infections (NBSI) during rotavirus outbreaks. We describe spectrum of bacterial pathogens and rate of NBSI prior to and during two separate outbreaks of rotaviral gastroenteritis.

**Method:** Hospital and laboratory databases were searched for positive NNU blood cultures during each rotaviral outbreak period and for a corresponding “control” period one year prior to each outbreak.

**Results:** The most prevalent bacterial pathogen isolated during all periods was *Klebsiella pneumoniae*, with predominance of extended spectrum B-lactamase producing (ESBL) strains. Other pathogens isolated included *Pseudomonas aeruginosa, Acinetobacter baumannii, Serratia marcesens, Enterobacter cloacae* and Methicillin-resistant *Staphylococcus aureus*. NBSI rates were similar during the control and rotaviral gastroenteritis outbreak periods (4.9 versus 5.1 episodes per 1000 patient-days). However, the proportion of NBSI due to *K. pneumoniae* increased significantly during the rotaviral outbreak periods, whilst other bacterial isolates declined. (p = 0.0001)

19. Rifampicin monoresistant *Mycobacterium tuberculosis* disease among children in Cape Town, South Africa

**Background:** The incidence of drug-resistant TB disease is rising worldwide. Although rare, rifampicin mono-resistant *Mycobacterium tuberculosis* (RMR-TB) has been increasingly reported particularly among adult HIV-infected populations. RMR-TB poses a significant threat to TB control programs as it is associated with extended treatment duration, elevated therapeutic cost, higher mortality and treatment failure rates. Paediatric RMR-TB has not previously been described in an HIV- and TB-endemic

**Method:** Records of children with culture-confirmed RMR-TB between 1 March 2003 and 28 February 2009 were identified from a prospectively-recorded database of drug-resistant TB at Tygerberg Children’s Hospital (TCH) and Brooklyn Chest Hospital (BCH), Cape Town, South Africa. Mutation analysis was performed on available specimens.

**Results:** Eighteen children with a median age of 6.9 years (range 2 months – 12.8 years) were identified. Nine (50%) were HIV-infected and 4 (22%) were HIV-exposed, uninfected. Eleven (61%) had previous TB treatment or prophylaxis. Nine children (50%) had cavitary disease. Five children (22%) had extrapulmonary disease. Twelve (67%) had adult TB source cases, including 5 (42%) adults with known RMR-TB. Primary transmission occurred among ten children (56%) and acquisition of RMR-TB was possible in 8 (44%) with prior TB treatment. Median delay to specific RMR-TB treatment was 70 days (range 2-188). One child died from RMR-TB meningitis. Gene mutations consistent with RMR-TB were confirmed in 5 available samples.

**Conclusion:** RMR-TB disease is increasingly encountered, particularly among HIV-infected and HIV-exposed uninfected children. Delay in commencing appropriate therapy for RMR-
TB and high rates of cavitary disease could be a source of RMR-TB transmission.

20. Zygomycosis mimicking a soft tissue tumour: a case report

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Background: Zygomycosis describes a group of fungal infections caused by pathogenic moulds belonging to the class Zygomycetes. It is a rare but important infection with symptoms that may be suggestive of a malignancy.

Case report: A 40-month old female patient presented with a 6-month history of progressive abdominal distension, fever and weight loss. She was chronically ill and wasted with generalised lymphadenopathy. On abdominal examination there was a firm irregular mass, measuring 10 cm × 5 cm, in the right hypochondrium extending to the right flank. Her HIV-ART was negative and the tuberculin skin test non-reactive. The complete blood count showed a marked thrombocytosis of 1731 × 10⁹/L and an elevated white cell count of 24 × 10⁹/L with a predominant lymphocytosis and no eosinophilia. A computed tomography scan of the abdomen showed inflammation and oedema of the right abdominal wall and omentum with enlarged intra-abdominal lymph nodes. Tissue sampling revealed marked fibrotic tissue with numerous eosinophils and giant cells. Bone marrow aspiration and PCR gene rearrangement studies were normal. An additional Grocott stain of the biopsy revealed occasional fungal elements suggestive of a subcutaneous phycymycosis. The galactomannan assay was negative with a positive (1,3)-beta-D-glucan assay of 102 pg/ml. A mouse anti-Zyomyceses antibody test was positive, confirming a diagnosis of Zygomycoses. Immune work up was normal. Initial administration of broad spectrum antibiotics had no effect. After starting treatment with Amphotericin B she responded rapidly with resolution of systemic symptoms as well as near complete resolution of the abdominal mass.

Discussion: Zygomycetes are filamentous fungi with a worldwide distribution. Cutaneous zygomycosis is one of the five major clinical presentations of this infection. A definitive diagnosis is made by histopathological examination with or without isolation of the fungus from the same site. Amphotericin B continues to be the mainstay of medical treatment.

21. Background of new subtypes and variants of hepatitis C virus genotype 4 in South Africa

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Background: Antimicrobial susceptibility surveillance of urinary tract infection (UTI) pathogens is important for appropriate empirical treatment. This study aimed to (i) assess the pathogens and susceptibility profiles of bacteria in the urine isolated from female patients with UTI symptoms in Gauteng and (ii) to describe empirical antimicrobial prescribing practice.

Method: Between December 2010 to June 2011, 196 female patients with UTI symptoms were enrolled at six sites within Gauteng. Participants were asked to provide mid-stream urine (MSU) samples. The STIRC laboratory (NICD/NHLS) performed culture and susceptibility testing. Only bacteria from urines yielding ≥10⁵ CFU/ml of bacteria were further investigated. For this analysis, isolates were categorised according to their Gram morphology (GNB = Gram negative bacillus; GPC = Gram positive coccus). Minimum inhibitory concentrations (MICs) were determined for quinolones (ciprofloxacin, norfloxacin...
and levofloxacin), cephalosporins (cefuroxime and cefixime), amoxicillin/clavulanic acid and co-trimoxazole by the E-test method (AB bioMerieux, Sweden).

**Results:** A total of 100 significant bacterial isolates were obtained from 98 women (2 women had dual infections). Antimicrobial prescribing data were available for 96% (94/98) of cases. A total of 63% (59/94) of patients received a fluoroquinolone, 16% (15/94) amoxicillin/clavulanic acid, 12% (11/94) fosfomycin and 5% (5/94) a cephalosporin; 4% (4/94) of patients were not treated empirically. The majority of the pathogens were Gram-negative (83%; 83/100); 17% (7/100) were Gram-positive species (E. faecalis, n=6; S. agalactiae, n=5; S. saprophyticus, n=5; S. haemolyticus, n=1). The most active agents against the Gram negatives were cefixime (93%) and the fluoroquinolones (93%). Amoxicillin/clavulanate was the most active agent against Gram-positive pathogens (100%). Cotrimoxazole resistance amongst Gram-negative and Gram-positive pathogens was 55% and 89%, respectively.

**Conclusion:** The majority of our patients received a fluoroquinolone empirically. The predominant pathogens cultured were Gram-negative pathogens. The majority of these strains were susceptible to the fluoroquinolones (ciprofloxacin, levofloxacin, norfloxacin) and the cephalosporins (cefixime, cefuroxime).

### 23. Management of an HIV-discardant mother and child

**Background:** Unexplained HIV-infection of an infant of an HIV-negative mother is a source of great anxiety and several possible modes of transmission have been described to date, including nosocomial infections, premasturbation of food, shared breastfeeding and mislabelling of breastmilk bottles among others. These modes of infection lead to postnatal infections with the opportunity to observe the rarely described acute seroconversion syndrome in the infant.

**Case report:** A 10 week old infant was hospitalized in severe respiratory distress. She was also anaemic, cyanosed and had axillary lymphnodes. Both the HIV ELISA and PCR tests were positive whereas the mother tested negative. Further investigations revealed a cytomegalovirus (CMV) pneumonitis, from which she recovered. Antiretroviral treatment was promptly started. Possible modes of HIV transmission were further investigated. The baby had been born in a midwife unit of a municipal clinic and transferred to a secondary hospital. She was treated for transient tachypnoea of the newborn for 6 days, which included intravenous fluids and medication. Later the mother gave a history of her sister having breastfed the child intermittently from 6 weeks of age. HIV sequencing and phylogenetic analysis provided evidence that the surrogate breastmilk was the source of the infant’s HIV infection.

**Discussion:** From the several available management options to investigate the possible causes of HIV discordance in this mother and child (nosocomial misadventures or even baby switching during the post-neonatal admission), phylogenetic analysis was available and led to the reassurance of the mother and the health care institution. Furthermore, the child presented with acute seroconversion syndrome complicated by a CMV pneumonitis, which is uncommonly described in children. This case re-emphasizes the risk of shared breastfeeding in a country with a high HIV prevalence and the value of having found a cause of the discordant HIV results between mother and infant.

### 24. Strongyloides hyperinfection treated with veterinary parenteral ivermectin

**Background:** Strongyloides stercoralis hyperinfection syndrome is an often fatal emerging infectious disease occurring typically when host immunity is impaired. It is uncommon in AIDS.

**Case report:** A 27-year old pregnant woman with HIV infection initiated tenofovir, lamivudine and nevirapine at a CD4 count of 58/μl. After 2 months on ART and at 28 weeks of pregnancy, she developed vomiting and abdominal pain ascribed to pregnancy. She was hospitalized in labour at 33 weeks, delivered a live infant, and was discharged day 2 post-partum despite vomiting. She was hospitalised 4 days later with vomiting, abdominal pain and pyrexia. The diagnosis was pupeeral sepsis which ultimately resulted in hysterectomy. Post-operatively she developed respiratory distress with bilateral infiltrates on X-ray and ileus. Sputum showed nematode filariform larvae and hookworm. Albendazole and systemic antibiotics were initiated. The patient deteriorated further. Up to 8 litres a day was lost in vomitus and diarrhoea. Abdominal CT revealed severe pancolitis and enteritis. Stool contained filariform larvae of *S. stercoralis* and hookworm ova. On day 20 post-hysterectomy, a diagnosis of *Strongyloides* hyperinfection was made. Albendazole was administered orally and rectally without response. Oral ivermectin was unavailable. Nosocomial septicaemia with *Enteroceoccus faecium* was treated with vancomycin. On day 25, veterinary parenteral ivermectin, given subcutaneously on alternate days for 21 days, was started with patient consent. On ivermectin day 3, vomiting stopped and by day 14 the diarrhoea had resolved. On day 49 post-surgery, the patient was discharged having restarted ART and remains well a year later.

**Discussion:** This is likely a case of IRIS, developing after ART initiation. Pregnancy and delivery complicated the course. Oral ivermectin, the drug of choice, is not registered in South Africa. There is no human parenteral treatment. The use of veterinary parenteral ivermectin, described in sixteen case reports, can be life saving.
25. The immunological detection of *Chlamydia trachomatis* and its association with preterm delivery

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**Background:** *Chlamydia trachomatis* is a common sexually transmitted disease (STD) worldwide. Since it is commonly asymptomatic in females it can result in complications for mother and infant during pregnancy. Although transmission of the organism is from mother to infant generally occurs during delivery the possibility of intrauterine infection via the ascending route at late pregnancy can occur. If left untreated this could have serious consequences for both mother and infant.

The objective of the study is to compare blood samples from full-term delivery (FTD, i.e. controls) with samples from preterm delivery (PTD, i.e. cases) for IgG and IgM in order to establish whether a correlation exists between PTD and Chlamydia infection.

**Method:** Maternal and foetal cord blood samples were collected from mothers attending the Obstetrics and Gynaecologic Unit of the teaching hospital in Butare, Rwanda. Samples were screened for *Chlamydia trachomatis* IgG and IgM with an ELISA assay using commercial kits.

**Results:** In the maternal FTD group (n=80), 7 samples were positive for IgG and 8 samples were positive for IgM. In the maternal PTD group (n=80), 8 samples were positive for IgG and 1 was positive for IgM. Of the FTD foetal cord samples (n=80) screened, 4 were positive for IgG and none was positive for IgM. Foetal cord blood in both groups showed that for both PTD and Chlamydia infection.

**Conclusion:** The results obtained indicate that within this population group *Chlamydia trachomatis* infection is not the major cause of PTD, but does imply the possibility of other microbial agents being the cause of PTD.

26. The correlation of blood and nasal Staphylococcus aureus isolates in patients admitted to the Tygerberg academic complex

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**Background:** *Staphylococcus aureus* has many virulence factors and the ability to adapt to different environments. It colonizes the skin and the nose of about 50% of the healthy population. Research in developed countries established a strain correlation between nasal and blood *S. aureus* strains of around 80%, and it was established that carriers are three times more vulnerable to bloodstream infection than non-carriers. Nasal colonization is considered a predisposing factor to *S. aureus* infections, including bacteraemia. The aim of this study was to examine this correlation in the setting of Tygerberg Hospital, a large academic hospital in South Africa.

**Method:** Patients with blood stream isolates of *S. aureus* were prospectively enrolled and screened for nasal colonization.

The strain relatedness between blood culture and nasal isolates was established by spa typing and pulse field gel electrophoresis (PFGE). Other genetic characteristics like agr group, SCCmec type and the presence of the pvl virulence gene were also investigated for 226 *S. aureus* isolates that were collected prospectively from 111 patients.

**Results and conclusion:** In this study 91 of 111 (82%) cases with *S. aureus* blood stream isolates (BSI) recorded to date were nasal carriers while 20 (18%) were not colonized. According to PFGE pattern analysis a nasal isolate similar to the BSI was found in 69 patients, establishing the colonizing strain as a likely source of infection in 62% of our cases. Of the 42 patients without a corresponding nasal strain, 22 were colonised with other strains of *S. aureus*. Forty five of the 111 cases were due to MRSA (40.5%). Of the colonised cases 33/69 (48%) were infected with MRSA, compared to 30/42 (71%) of non-colonised cases. This difference was statistically significant (p value of 0.015) indicating a greater probability of MRSA with exogenous infection. Further studies will investigate the association of genetic and functional virulence factors with colonisation and invasion.

27. Bicytopenia due to *Mycobacterium avium* immune reconstitution inflammatory syndrome

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**Background:** Over 90% of the acid-fast bacilli (AFB) seen on Ziehl Neelsen (ZN) stain in South Africa are due to tuberculous mycobacteria, with non-tuberculous mycobacterial disease accounting for less than 10% of disease. As a result, almost every positive result for AFB is presumed to be due to *Mycobacterium tuberculosis*. In the face of chest symptoms, associated B-symptoms (fever, weight loss, night sweats) and suggestive chest X-ray, this presumption seems very reasonable, and treatment is usually commenced. Human immunodeficiency virus (HIV) infection has led to an increase in cases of tuberculosis. However, there has also been an increase in opportunistic non-tuberculous mycobacterial disease. *Mycobacterium avium (M. avium)* is one such pathogen whose risk increases as the CD4+ cell count decreases.

**Case report:** We present a case of a 44-year-old male with advanced HIV disease (CD4+ count 85 cells/mm³) who had been started on ART one month prior to presentation. He had a history of post tuberculous bronchiectasis and previous Cryptococcal meningitis. He was referred for persistent bicytopenia with haemoglobin 6.8g/dl and platelet count 4x10 /l. His only symptom was loss of weight. Bone marrow investigation revealed a hypercellular marrow with areas of fibrinous myelitis and caseous necrosis. No formed granulomata were seen. ZN stain was positive for acid fast bacilli. Bone marrow culture yielded *M. avium*.

**Discussion:** Disseminated *M. avium* disease occurs almost exclusively in patients with advanced HIV disease, and
may present as an immune reconstitution inflammatory syndrome (IRIS) after commencement of antiretroviral treatment (ART). *M. avium* may present with symptoms typical of tuberculous. The yield for *M. avium* is greatest in blood and bone marrow cultures. *M. avium* disease should form part of the differential diagnosis in advanced HIV disease. IRIS may contribute to the severity of cytopenias and particularly to thrombocytopenia.

28. Chronic strongyloidiasis with hyperinfection: an institutional threat

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**Background:** Strongyloides stercoralis, a parasitic soil nematode, has an estimated prevalence of 50-100 million infections worldwide, mostly in tropical and sub-tropical regions. It is one of the few helminths that can reproduce in life with adult seroprevalence approaching 100% by early childhood. It is one of the few helminths that can reproduce in the human intestine, and recognize the potential for nosocomial transmission.

**Case report:** We describe an institutional case of chronic strongyloidiasis with hyperinfection syndrome (HS). The patient, a 14-year-old boy, presented with a history of chronic diarrhoea, vomiting and loss of appetite. He was severely wasted (marasmic), mildly pyrexial and had features of protein-losing enteropathy. Full blood count showed a peripheral eosinophilia with a raised absolute eosinophil count. Microscopic examination of concentrated stool revealed large numbers of viable rhabditiform and filariform larvae of *Strongyloides stercoralis*. He was commenced on oral albendazole therapy and eventually discharged in a stable condition.

**Discussion:** Strongyloides is an emerging global infection and a major public health concern, particularly with the increasingly widespread use of immunosuppressive therapies. Malnutrition, exacerbated by chronic *Strongyloides* diarrhoea, may trigger HS. This aggressive, fulminant and often fatal syndrome may have relatively non-specific gastro-intestinal manifestations. Peripheral eosinophilia is frequently absent in immunosuppressed hosts with overwhelming infection. Clusters of cases in mental institutions have been well documented, and suggest the possibility of nosocomial transmission. Clinicians need to have an increased awareness of the risk factors for HS, and recognize the potential for nosocomial transmission. Novel molecular high-throughput detection methods could replace relatively-insensitive stool microscopy. Preventive efforts should be geared towards screening at-risk hosts for chronic infection, and the establishment of geo-helminthic control programs in endemic regions.

29. Evaluation of an automated *Treponema pallidum* immunoassay as a screening test for syphilis

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**Background:** Globally, and within Southern Africa, syphilis is the second most frequent cause of genital ulcer disease and remains an important cause of adverse pregnancy outcomes. For reasons of economy and convenience, high volume clinical laboratories have begun screening using automated treponemal tests. There is a reversal of the testing sequence: initial screening with a specific treponemal test, and subsequent testing of reactive results with a nontreponemal assay (rapid plasma reagin / RPR).

We evaluated the performance characteristics of the Abbott Architect™ TP, a chemiluminescent microparticle immunoassay, as an automated screening test for syphilis.

**Method:** Eighty five consecutive serum specimens, including some stored RPR positive specimens, were tested using the Architect TP. All specimens underwent concurrent testing with RPR (normal screening test for syphilis) and TPHA (confirmatory specific treponemal assay). Specimens giving discordant results were tested using a third specific treponemal assay (*Treponema pallidum* particle agglutination assay/ TPPA), considered the gold standard test.

**Results:** Twenty six specimens were Architect TP reactive and 59 were non-reactive. The sensitivity and % correlation of Architect TP in comparison to the RPR were 85.7% and 92.9%, respectively. Retesting of discordant specimens with TPPA revealed three false positive RPRs, and one false negative Architect TP.

The specific treponemal assays were compared with each other. The sensitivity, specificity and % correlation of the Architect TP compared to TPHA were 92%, 96.6% and 95.3%, respectively. Against the TPPA, sensitivity, specificity and % correlation increased to 96.3%, 100% and 97.6%, respectively.

**Conclusion:** The automated Architect TP assay compares favourably with the gold standard TPPA and is an appropriate screening test in high volume laboratories. It obviates the need for manual screening and has the additional advantage of eliminating non-specific reactivity. All Architect TP reactive specimens must undergo further testing with a nontreponemal assay to identify active, untreated infections.

30. Severe cytomegalovirus pneumonia in an immunocompetent paediatric patient

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**Background:** Cytomegalovirus (CMV) infection occurs early in life with adult seroprevalence approaching 100% by early adulthood in developing countries. Primary CMV infection does not always cause clinical illness in healthy children. However, a severe life threatening infection may occur in both
immunocompetent and immunocompromised individuals. We report a case of severe CMV pneumonia in immunocompetent paediatric patient.

Case report: 11 month old female was admitted to Dr George Mukhari hospital with one week history of cough, breathing difficulties, fever and convulsion. On arrival, baby was in respiratory distress and unconscious. Temperature was 39˚C and heart rate was 170/min. Respiratory rate was 64/min and oxygen saturation was 53% which was only to 58% on 3 LO2/min. Patient was malnourished with less than <3rd percentile of weight for age. Bilateral crepitations on lung fields, hepatomegaly and hypotonia were noted. No meningeal signs. She was ventilated and intravenous ceftriaxone was commenced. Investigations for severe pneumonia, kwashiorkor and meningitis were performed. Chest X ray showed bilateral diffuse infiltration. CSF, nasopharyngeal aspirate (NPA) and blood culture for bacteriological investigations were negative. HIV-PCR was also negative. CMV DNA was detected in plasma and NPA specimens with viral load 997 copies/ml (3.0 log10) and 252000cps/ml (5.4 log10) respectively. Patient continues to desaturate on high setting ventilation and demised after 3days in ICU before commencing ganciclovir.

Discussion: This case highlighted the fact that CMV can cause serious complications in children regardless of immunological status. The existing danger of CMV infection in children should not be underestimated. CMV should be considered as a cause of serious complications in children regardless of immunological status.


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Background: South Africa experienced a measles outbreak in 2009-2010 with more than 17 000 confirmed cases reported by the National Institute of Communicable Diseases. We studied the impact of the outbreak on a paediatric hospital in Cape Town.

Method: Children with measles at Red Cross Children’s Hospital from November 2009 to July 2010 were retrospectively identified from hospital admissions data and notification records. Basic demographic information was obtained for outpatients; inpatient data was captured in detail.

Results: In total, 1809 children with measles were seen over 9 months. Median age was 8.5 months (interquartile range (IQR) 5.7 to 22.0 months). Vaccination status was poorly recorded. Of the 911 children eligible for measles vaccine, 397 (43%) had vaccination status documented (185 (20%) had had at least 1 dose of measles vaccine; 212 (23%) had never been vaccinated). There were 537(30%) admissions; 284 (53%) were males. The most common reason for admission was pneumonia (356, 66%) and/or diarrhoea (259, 48%). The median age of those admitted was 7.6 months (IQR 4.9 to 11.5 months). Of the 34 HIV-infected children, 20 (59%) were on anti-retroviral therapy (ART) at the time of measles diagnosis; although 22 were eligible for vaccination, only 3 (14%) had documented evidence of measles vaccination. Seven children died (case fatality 0.4 per 100 diagnosed). All were younger than 1 year; 2 (29%) were HIV-infected; both were on ART. Only 1 of the children who died had had measles vaccination. Six (86%) of the deaths were females.

Conclusion: The highest burden of disease (admissions and deaths) was amongst children under 1 year of age. Despite poor recording of vaccination status, we observed a large number of vaccine failures. Case fatality was low, but females were over-represented.

32. Excellent long-term safety of isoniazid preventive therapy in children with HIV: a randomised controlled trial comparing two dosing schedules

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Background: Isoniazid preventive therapy (IPT) significantly reduces tuberculosis incidence in HIV-infected children. Risk for IPT-related liver injury has not been defined for this population.

Method: HIV-infected children aged ≥8 weeks enrolled in a placebo–controlled, randomized trial of IPT, given daily or thrice weekly, in Cape Town, South Africa. The placebo arm was terminated early; isoniazid was given for up to five years. Alanine aminotransaminase (ALT) was measured at baseline, 6-monthly and during intercurrent illness. Severe liver injury was defined as a ≥ 10-fold rise in ALT. Risk factors were assessed with incidence rate ratios (IRR) and hazard ratios from Cox proportional hazards regression.

Results: Of 324 children enrolled, 159 (49%) were randomized to the daily arm. Most were young [median age 23 months (interquartile range, IQR 9.5-48.6)] and/or immuno-compromised [median baseline CD4% 20% (IQR 13.6-26.9)]; 207 (63.9%) received highly active anti-retroviral therapy (HAART). Median baseline ALT was 28 U/l (IQR 18-42). There were 19 episodes of severe liver injury: 16 occurred on IPT [incidence rate (IR) 2.86 per 100 child-years], compared to 1 on placebo [IR 1.7 per 100 person-years; IRR 1.68 (95% CI 0.3-70.6)]. Two episodes occurred during TB treatment. Children receiving IPT with HAART had similar rates of severe liver injury compared to those receiving HAART only [IRR 0.29 (95% CI 0.39– 12.88)]. Younger age [adjusted hazard ratio (aHR) 0.66 (95% CI 0.46-0.94), p=0.02] and higher CD4% [aHR 1.06 (95% CI 1.02-1.12) p=0.008] were risk factors. Fourteen (74%) of 19 events were unrelated to IPT: other causes included viral hepatitis (8), abdominal tuberculosis (1), HAART (3) and TB treatment (2). The incidence of IPT-related liver injury was 0.78 per 100 person-years. One child died of an unrelated cause, none had hepatic failure. All surviving children were successfully restarted on IPT.

Conclusion: Prolonged IPT is safe in HIV-infected children.
33. Microbe of the month surveillance tool kit

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Background: Health-care-associated infection is the most frequent result of unsafe patient care worldwide, but there is a shortage of data available from the developing world. In the Southern African context, operational challenges such as nursing and healthcare worker skills shortages, inappropriate antimicrobial usage, multidrug or pan-resistant microorganisms and HIV related conditions make the practice of infection prevention and control more important than ever. Unfortunately, the often unpopular rôle and responsibility as ‘Enforcer’ usually falls to the Infection Control Nurse – which in the private hospital setting, may not promote problem oriented thinking and multidisciplinary collaboration for undertaking proactive measures at unit level. And so the idea for a ‘fresh approach to a tired topic’ was born – a monthly surveillance and awareness tool kit, compiled by the Infection Control Nurse (ICN) - summarizing unit specific information for nursing staff, critical care physicians and allied healthcare professionals.

Method: The objective was to present facts on the prevalence of significant pathogens cultured from ICU patients, basic statistics regarding healthcare associated infections and infection rates, as well as an educational and visually appealing ‘microbial fact sheet’ on a relevant micro-organism. This information was displayed inside an A3 frame, in plain view, and updated monthly by the ICN.

Results: The concept of introducing a ‘Top 10 Hit Parade’ was met with interest and amusement; and it was not long before staff and doctors began to take an active interest in the project! It also created a useful overview of antimicrobial usage in the unit, which had previously not been given much consideration.

Conclusion: ‘Microbe of the Month’ was subsequently expanded to the cardiac and neonatal ICU’s, and proved to be a popular educational inclusion in infection control committee meetings and Link Nurse continuing professional development.

34. Colistin antimicrobial susceptibility testing methods for Acinetobacter baumannii and Pseudomonas aeruginosa isolates: a comparison between broth microdilution and Etest®

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Background: Multidrug resistant (MDR) Acinetobacter baumannii and Pseudomonas aeruginosa are a growing threat, necessitating the increased use of colistin. Subsequently there is a need for laboratories to provide accurate and reliable antimicrobial susceptibility testing (AST) of colistin. Unfortunately AST of these isolates is problematic with standardization bodies stipulating that minimum inhibitory concentration (MIC) methods be used. The Etest® is an attractive option given its ease of use. Based on our experience of difficulties in interpreting the colistin Etest®, we compared the MICs generated by broth microdilution (BMD) with those of the Etest®.

Method: MDR strains of Acinetobacter baumannii and Pseudomonas aeruginosa from various sites were collected from Johannesburg tertiary hospitals. Colistin sulphate pharmaceutical grade powder was used for determination of MICs by the BMD method, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Etests were performed concurrently and all MICs read by at least 2 observers. Comparative analysis was done by interpretive/ categorical agreement (CA) and actual MIC/ essential agreement (EA).

Results: A total of 62 isolates were tested, including 30 sterile site isolates. EA was only 26.3% (10/38) and 20.8% (5/24) for the A. baumannii and P. aeruginosa isolates, respectively. The CA for the P. aeruginosa isolates was only 25% (6/24), whilst 4 very major errors were detected for the A. baumannii isolates. Only 3 colistin resistant A. baumannii isolates were detected by BMD.

Conclusion: These results suggest that the Etest®, although convenient and relatively easy to perform, is not a reliable method for determination of colistin MICs in these nonfermentative microorganisms. We believe this warrants further evaluation on a broader range of isolates and across different AST platforms.

35. Comparative evaluation of a generic piperacillin-tazobactam compound by broth microdilution minimum inhibitory concentration testing

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Background: After almost 20 years of use in this country, piperacillin - tazobactam is still widely used in the treatment of healthcare – associated infections. Susceptibility ranges regionally from 8 to 97% for public sector blood culture isolates of Pseudomonas aeruginosa and Acinetobacter baumannii. A generic piperacillin-tazobactam is available and in the context of increasing resistance, diminishing therapeutic options and a resource limited setting, continued surveillance of piperacillin-tazobactam susceptibility and microbiological evaluation of generic substitutes is important. We report on our findings from an evaluation of a generic piperacillin-tazobactam (Tazobax®) using gold standard antimicrobial susceptibility testing (AST) methodology.

Method: Clinically relevant sterile site isolates were collected from a tertiary hospital in Johannesburg. Minimum inhibitory concentration (MIC) testing was done by broth microdilution. All isolates were tested simultaneously against both the generic and innovator piperacillin-tazobactam compounds, maintaining an 8:1 ratio of piperacillin to tazobactam throughout the dilution series. Comparative assessment of MICs was done using categorical (based on interpretive breakpoints) and essential (based on actual MIC) agreement. Error rates were calculated for very major (VME), major (ME) and minor errors (mE).
**Results:** A total of 140 isolates were tested, including 52 members of the *Enterobacteriaceae* family, *Pseudomonas aeruginosa* (20), *Acinetobacter baumannii* (47), *Staphylococcus aureus* (10) and *Enterococcus* spp. (11). Overall categorical agreement and essential agreement were 94.3% and 95.0% respectively. There were no VME or ME, with a 5.7% ME rate. Review of reported susceptibility data for the *A. baumannii* isolates, as per the laboratory information system, indicated a high level of interpretive discordance with the reference broth microdilution MICs (21.3%).

**Conclusion:** By reference method MIC testing, we have demonstrated microbiological equivalence between a generic piperacillin-tazobactam and the innovator product. Further evaluation of AST methods for piperacillin-tazobactam against *A. baumannii* isolates is warranted.

36. Broth microdilution antimicrobial susceptibility testing of enterococci against linezolid

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**Background:** Linezolid has been in use in South Africa since May 2002. It is primarily reserved and used for the treatment of resistant gram-positive organisms, especially vancomycin resistant enterococci (VRE). To our knowledge there are no published reports of linezolid minimum inhibitory concentrations (MICs) for enterococci. We evaluated the linezolid MICs of clinically significant enterococcal isolates (including VRE) from Johannesburg tertiary hospitals. Furthermore we assessed the MICs of a subset of VRE (*Enterococcus faecium*) to ampicillin.

**Method:** We collected 55 isolates, from various specimens, of *Enterococcus faecium* and *Enterococcus faecalis*. Linezolid MIC testing was done by the broth microdilution method, as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Ampicillin MICs were determined using the Etest™ method.

**Results:** Linezolid MICs were performed on 41 *E. faecium* isolates and 14 *E. faecalis* isolates. Thirty VRE isolates (*E. faecium*) were included. The MIC<sub>50</sub> and MIC<sub>90</sub> was 2µg/ml and 4µg/ml respectively, with no difference between VRE and vancomycin susceptible enterococcal isolates. Ampicillin MICs for a subset of 20 VRE isolates was >256µg/ml.

**Conclusion:** We have shown that the linezolid MICs of enterococci in our setting are high. The susceptible breakpoint according to CLSI is 2µg/ml. A MIC<sub>90</sub> of 4µg/ml means a significant number of isolates are in the intermediate category. This coupled with high level ampicillin resistance potentially limits treatment options for VRE. Continued surveillance to detect emerging resistance and review of current dosing regimens to prevent emergence of resistance is warranted. Surveillance of isolates from other regions and sectors is necessary to establish what the situation is on a national scale.

37. A review of microorganisms associated with systemic infections in a burns unit at a tertiary level hospital in KwaZulu-Natal

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**Background:** Patients that suffer severe burns are at a higher risk for local and systemic infections, characterized by high morbidity and mortality. The commonest micro-organisms of sepsis in burns still remain *Staphylococcus aureus* including MRSA and *Pseudomonas aeruginosa*. However MDR organisms including *Pseudomonas aeruginosa* and *Acinetobacter spp*. as well as emerging unusual organisms including fungi have contributed to increase mortality rates. Empiric antibiotic is often initiated in the setting of invasive infections therefore the common organisms and their antibiogram should be established to further direct empiric therapy. The aim of this study is to determine the predominant as well as unusual organisms causing systemic infections in the unit specifically.

**Method:** We did a statistical analysis of micro-organisms causing systemic infections in burns unit for the duration January 2010-April 2011.

**Results:** 95 blood cultures came up positive with microorganisms. 92 out of 95 were significant pathogens. Among these, 79 % (75/95), 18% (17/95), 3 % (3/95) for Gram-negative, Gram-positive, contaminated blood cultures respectively. Of the gram negatives: *Acinetobacter spp* - 37 % (28 / 75) and of the gram positives: *S. aureus* 47% (8/17). The rest details will be shown as graphs in poster.

**Conclusion:** *Acinetobacter spp*. was the leading cause of all micro-organisms causing bacteraemias in the burns unit. *S. aureus* still remains the predominant gram positive. *Acinetobacter spp*. has emerged as the commonest cause of bacteraemias followed by *Klebsiella pneumonia*. Blood culture contamination rate was low. Collistin has re-emerged as a highly effective antibiotic against MDR *Acinetobacter spp*. Appropriate antibiotic therapy contributes to a significant reduction in morbidity and mortality rates in burns patients therefore prompt reporting of positive blood cultures with directed antibiotic therapy is of paramount importance.

38. Antifungal susceptibility profile of yeast isolates from sterile sites at a tertiary hospital in South Africa

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**Background:** Invasive infections caused by yeasts are associated with high mortality and morbidity and resistance to antifungal agents is increasing. Amongst other yeasts, *Candida* spp. have emerged the leading cause of nosocomial bloodstream infections.

**Method:** Yeast isolates from sterile body site specimens were identified to species level using germ tube test and ID 32C API kits. The in-vitro antifungal susceptibility testing to fluconazole and voriconazole was performed by disc diffusion method in accordance with the CSLI guidelines.

**Results:** The distribution of yeast isolates in this study was as follows: *C. krusei* (41.5 %), *C. albicans* (32.0%), *C. inconspicua*
C. albicans in both isolates were resistant. One isolate of adenovirus can infect many organs, CNS infection is rare. Although gastroenteritis and respiratory infection in children.

Discussion: The patient recovered after 2-days with reversal of neurological signs. The patient was referred from a district hospital for first episode of acute encephalopathy associated with adenovirus as an aetiological agent of acute convulsions and encephalopathy. We report a case of acute encephalopathy associated with adenovirus.

Case report: This is a case of a 20 month old baby who was referred from a district hospital for first episode of status epilepticus. The patient was acutely ill with decreased level of consciousness without signs of meningitis. The patient was treated for acute diarrhoea a week prior to development of convulsions. Blood urea and electrolytes, electroencephalogram and CAT-scan were also normal. CSF chemistry and bacteriological findings were normal. CSF PCR for herpesvirus and enterovirus were negative. However adenovirus was isolated from cell culture inoculated with CSF. The patient recovered after 2-days with recovery of alertness and reversal of neurological signs.

Discussion: Adenovirus is mostly found in cases of gastroenteritis and respiratory infection in children. Although adenovirus can infect many organs, CNS infection is rare.

Adenovirus should be considered as one of the viral causes of acute encephalopathy and convulsions in children.

40. Detection and characterisation of ESBL-positive Klebsiella pneumoniae clinical isolates

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Background: The emergence of extended-spectrum betalactamase (ESBL) production in K. pneumoniae is of great concern. Bacteria capable of ESBL-production complicate treatment options, because if detected the bacteria must be reported as resistant to all penicillins, cephalosporins and aztreonam regardless of susceptibility testing results. It is essential for diagnostic laboratories to use rapid and reliable methods for the detection of ESBL-producing bacteria.

Method: This study investigated the prevalence of ESBL-producing K. pneumoniae clinical isolates and determined the sensitivity and specificity of the Vitek2 automated system (bioMérieux, France) and multiplex PCR assay (targeting blaTEM, blaSHV and blaCTX-M genes) against the combination disc method in detecting ESBL production. A total of 150 consecutive clinical isolates of K. pneumoniae were collected and analysed for ESBL-production. The combination disc method was considered the gold standard; this method was performed and interpreted according to the Clinical and Laboratory Standard Institute (CLSI) 2009 guidelines.

Results and conclusion: The prevalence of ESBL positive K. pneumoniae according to the combination disc method (recommended by CLSI) was 57.4% (85/148). Compared to the combination disc method, which was used as a confirmatory test, the sensitivity and specificity of the Vitek2 system in detecting ESBL-production was 99% (84/85) and 98% (62/63) respectively. The sensitivity and specificity of the multiplex PCR assay (using blaTEM as a true marker for ESBL-production) was found to be 96% (82/85) and 97% (61/63) respectively. There is a high prevalence of ESBL-producing K. pneumoniae isolates in the Tshwane area. Infection control measures to reduce the spread of ESBL-producing isolates are recommended. A good correlation was obtained between the three assays (Multiplex PCR assay; Vitek2 automated system and the combination disc method) evaluated in this study.

41. Direct identification of Gram-negative bacilli from blood cultures (Bact/Alert) by using the VITEK® 2 system

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Background: A pilot study was conducted utilising a direct inoculation method of positive blood cultures (charcoal bottles/FAN and non charcoal bottles) on the Vitek2 automated system. As this system can potentially identify gram negative bacilli in a minimum of 4 to 5 hours, this method should have a significant impact on turnaround time (TAT) resulting in better patient management.
Method: Inocula were limited to specimens that were read as unimicrobial gram negative bacilli on the Gram stain and were prepared according to a specified in house centrifugation method. These were then inoculated onto the Vitek cards as per manufacturer’s specifications. Results were compared to those obtained from conventional method of identification, the API Identification system for Enterobacteriaceae and other non-fastidious gram negative rods.

Results: A total of 25 blood cultures were processed (20 charcoal and 5 non charcoal bottles). Concordance between the test method and the conventional method was 85 % (17/20 charcoal bottles) and 80 % (4/5 standard bottles).

Conclusion: Based on the above results, this method looks promising, especially for charcoal containing bottles, as a direct inoculation method. Further study is planned with larger specimen numbers and the possible inclusion of antimicrobial susceptibilities.

42. Characterisation of Ureaplasma parvum from symptomatic and asymptomatic men attending a family practice in Pretoria, South Africa

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Background: The genus Ureaplasma colonises human mucosal surfaces of urogenital tract of men and women. In men, it causes non-gonococcal urethritis and infertility. Although its pathogenesis is not yet fully understood, it has been suggested that certain serotypes are associated with disease. This study undertook to detect genital Ureaplasma species and to characterise Ureaplasma parvum in men with and without urogenital symptoms.

Method: 200 first void urine specimens were collected from symptomatic (100) and asymptomatic (100) men. All specimens were cultured in U9 broth and subcultured on A2 agar. All isolates were tested for susceptibility using the Mycofast Evolution 3 kit. DNA was extracted and amplified using a multiplex TaqMan PCR assay targeting the MBA gene for the detection and serotyping of U. parvum. Ureaplasma urealyticum was detected by a commercial real-time PCR kit.

Results: Cultures were positive in 16/100 symptomatic and 12/100 asymptomatic men. All isolates were susceptible to doxycycline, pristinamycin, roxycycline and azithromycin. One Ureaplasma spp. from an asymptomatic male was resistant to ciprofloxacin and josamycin and intermediately resistant to ofloxacin and another was resistant to ofloxacin. An isolate from a symptomatic man was resistant to ciprofloxacin. There was no significant difference (p=0.16) between the U. parvum isolated from symptomatic (11/100) and asymptomatic (18/100) men and U. urealyticum from symptomatic 16/100 and asymptomatic 15/100 men (p=0.86). The predominant serotype was 6, followed by 1, 3 and 14 with no significant difference between symptomatic and asymptomatic men (p=0.309).

Conclusion: There is no data of circulating U. parvum serotypes from South Africa. There was no significant differences between symptomatic and asymptomatic men for either ureaplasmas. Serotype 6 was the most type commonly detected to developed countries, which suggests type 3. Macrolides and tetracyclines remain effective drugs for Ureaplasma infections. Molecular techniques are valuable identification and characterization methods of fastidious group of bacteria.

43. The prevalence of laboratory-confirmed acute hepatitis A infection in Dr George Mukhari Hospital, North of Pretoria

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Background: Hepatitis A virus is one of the commonest causes of hepatitis globally. In Africa, >90% of population became exposed to the virus through foecal oral route by the age of 10. Although hepatitis A infection is mostly asymptomatic, many children require hospital admission due to hepatitis A associated severe gastroenteritis and jaundice. We investigated the prevalence of hepatitis A infected patients who required hospital admission.

Method: This is a descriptive study regarding hepatitis A infection at Dr George Mukhari hospital. The data were analysed from a laboratory surveillance record between June 2010 and June 2011 using MS Excel.

Results: Total 1421 patients were investigated for hepatitis serology during the study period and 40 patients were confirmed as acute hepatitis A infection by ELISA (2.8% prevalence). All of acutely infected patients, 30%(22/73) were younger than 10 years old, 3.3% (14/419) were between 10-30 years old and 0.4% (4/929) were older than 30 years of age. Mean ALT was 1041IU/ml (11-4871) and mean AST was 745.3IU/ml (16-3339). No specific seasonal distribution was noted for the hepatitis A infection.

Conclusion: The prevalence rate of acute hepatitis A infection was found to be 2.8% in the patients presented with gastroenteritis and jaundice which is similar to previous reports. Hepatitis A virus contribute 30% of gastroenteritis and jaundice in children younger than <10 years old. Non-immune cases of hepatitis A in adolescence and young adults are still at risk of acquiring severe acute hepatitis A infection. Improving economy and sanitation status might increase the non-immune adolescence and adult who in turn are at risk of suffering acute hepatitis A infection which may require hospital admission.

44. Evaluation of the Affirm® VPIII microbial identification test for the diagnosis of vaginitis and bacterial vaginosis

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Background: Diagnosis of vaginitis and bacterial vaginosis is based on clinical signs and symptoms, microscopy, pH and the “whiff” test, and laboratory performed tests. The Affirm® VPIII Microbial Identification test (Becton Dickinson) incorporates non-amplified DNA probes for Candida species, Trichomonas vaginalis (TV) and Gardnerella vaginalis. The objective was to compare the performance of the Affirm VPIII test to detect Candida species, Gardnerella vaginalis and TV to graded Gram
The Affirm® VPIII is an objective system which determined using the graded Gram staining as a gold standard for BV and Candida species and the real-time PCR for TV.

Results: The Affirm® VPIII gave a sensitivity of 98% for BV correctly categorizing 80% of the BV positive specimens and specificity of 76%. For Candida it yielded a sensitivity of 86% and specificity of 95%. Compared to the TV PCR the specificity was at 45% and specificity at 99%.

Conclusion: The Affirm® VPIII is an objective system which can be used in any setting. The performance characteristics of the test for BV and Candida were comparable to those of other published studies. However in this study the TV PCR was used as a gold standard therefore the sensitivity of the Affirm® VPIII of 45% is similar to that of culture or wet mount as expected.

45. Acute viral hepatitis A infection with severe hepatic and renal failure

Background: In Africa, 90% of children have exposure to the hepatitis A virus by the age of 10. Acute hepatitis A infection in early childhood is usually asymptomatic and serious complications in adult are rare. We report the case of acute viral hepatitis A infection causing sub-fulminant hepatitis and acute renal failure at Dr George Mukhari hospital.

Case report: 15 years old female newly diagnosed type 1 diabetes mellitus was transferred from level one hospital for liver failure and renal failure. Patient was apyrexial, jaundiced, with generalized anasarca and decreased level of consciousness. Diabetic ketoacidosis was excluded. Patient was transferred to ICU for ventilatory support. The laboratory investigation revealed creatinine (674 μmol/l), LDH (11625 mol/l), ALT (1320 U/l), AST (1214 U/l). Infection screening test revealed negative for HIV serology and other hepatitis viruses. Resistance to ciprofloxacin was 22% while 95% were susceptible to nitrofuratoin. Of the thirty isolates resistant to cotrimoxazole, co-resistance with ampicillin was 86% and 23% respectively. Of the forty one isolates confirmed as being Escherichia coli. Antimicrobial susceptibility testing showed that 87.8% and 80.5% were resistant to cotrimoxazole and ampicillin respectively. Resistance to ciprofloxacin was 22% while 95% were susceptible to nitrofuratoin. Of the thirty five isolates resistant to cotrimoxazole, co-resistance with ampicillin and ciprofloxacin was 86% and 23% respectively. Even though 22% isolates were resistant to ciprofloxacin, 22% were co-resistant with cefotaxime. This was the only isolate that was an ESBL producing organism 2%. No SHV genes were detected in the isolates tested. Of the 41 tested, 76% were found to carry the TEM gene. One isolate contained both the TEM and CTX-M gene and was found to contain an extended spectrum beta lactamase.

Conclusion: This study confirms the high prevalence of antimicrobial resistance to commonly used urinary antimicrobials in Escherichia coli isolated at this institution as well as concurrent resistance to other commonly used agents. TEM and CTX-M were the beta-lactamases identified in these isolates.
the Eastern Cape Province. Pitchford et al. (1960) found a prevalence of 14% in school children in the northern part of the coastal area around Port St Johns, with the distribution map showing a steady decline southwards to East London, which had a 1% infection rate. The aim of this study was to measure the prevalence of urinary schistosomiasis among schoolchildren of Hobeni Junior Secondary School in the Mbashe district of the Eastern Cape Province.

**Method:** Urine samples were collected from 209 children in October 2008. The urine was analysed by urine dipstix immediately and later by microscopy. For microscopy the samples were concentrated by gravitation and centrifugation methods and afterwards investigated by a novel method, the ‘Transkei slide’.

**Results:** Two hundred and nine samples were analysed. In 73.2% of the pupils, *Schistosoma haematobium* eggs were found in the urine. Macrohaematuria, as a sign of severe infection was seen in 32.1%. Hobeni is 100 km south-west of the Port St Johns area. A prevalence of 73.2% in our area is unexpectedly high and we can assume that there has been an increase in the prevalence of schistosomiasis in the coastal area of the Transkei since earlier studies. Climate changes, with the correlated distribution of the host snail and changes in water use behaviour are possible explanations.

**Conclusion:** Schistosomiasis lacks the attention it deserves in South Africa. With almost three in four children being infected in the investigated area, it cannot be ignored. Regular treatment campaigns are needed in high prevalence areas. Praziquantel needs to be available in sufficient amounts at primary healthcare level, to a similar extent as deworming agents. To develop treatment plans and goals, an assessment of the disease burden is therefore a public health priority.

48. The impact of antiretroviral treatment on cryptococcosis in the Free State: 2006-2010

**Background:** The HIV/AIDS & STI National Strategic Plan, launched in 2007, aims to provide antiretroviral treatment (ART) to 80% of the South African HIV-infected population by 2011. In 2008, national ART coverage was 40% for adults with a CD4+ T-lymphocyte count <200 cells/µL and only 26% in the Free State (FS). We aimed to look at the impact that the ART programme has had on the incidence of cryptococcosis, an AIDS-defining opportunistic infection, in the FS.

**Method:** Cases of laboratory-confirmed cryptococcosis from the FS were reported to a national laboratory-based surveillance programme (GERMS-SA) from 1 January 2006 through 31 December 2010. Clinical data were collected from patients at 2 enhanced surveillance sites (ESS). Incidence was calculated using Statistics South Africa population denominators.

**Results:** Between 2006 and 2010, 2108 cases of cryptococcosis were reported from the FS. Incidence of cryptococcosis increased from 10 to 18 per 100 000 persons from 2006-2008, followed by a marked decrease to 11 per 100 000 persons in 2010. Ten percent (211/2108) of cases were reported from an ESS; 94% (199/211) had clinical information available. Ninety-five percent (172/182) of patients with an available HIV result were HIV-infected, and 94% (144/153) with an available CD4+ T-lymphocyte count were severely-immunosuppressed (CD4+ T-lymphocyte count <200 cells/µL): 58% had a CD4+ T-lymphocyte count <50 cells/µL. Concurrent use of ART at the time of diagnosis of cryptococcosis rose steadily from 9% (2/21) in 2006 to 37% (11/30) in 2010.

**Conclusion:** Although the incidence of cryptococcosis in the FS steadily increased over the earlier years of the ART programme, by 2010, incidence of cryptococcosis had declined markedly. ART use amongst HIV-infected patients with cryptococcosis increased over the years, in keeping with provincial data on ART. Increased uptake of ART amongst HIV-infected patients will eventually reduce the burden of cryptococcosis in South Africa.

49. The prevalence and identification of beta-lactamase-producing *Klebsiella pneumoniae* isolates at the Dr George Mukhari Hospital laboratory, and the identification of their beta-lactamase genes

**Background:** The rapid development of resistance to the conventional antibacterial agents used for the common uropathogen, *Klebsiella pneumoniae*, has led to increased morbidity and mortality in infected patients. While it is well established that beta-lactamase production is the most common cause of decreased susceptibility, the prevalence and characterization of these beta-lactamase genes has not been well defined at Dr George Mukhari Hospital laboratory. The purpose was to phenotypically and genotypically characterise *K. pneumoniae* isolates at this institution and identify the prevalence of bla*SHV*, bla*TEM* and bla*CTX-M* genes.

**Method:** Forty six isolates were collected. The phenotype was confirmed by the API 20E test. The disc diffusion method was used for susceptibility testing.TEM, SHV, and CTX-M beta-lactamase gene families were identified by PCR using specifically designed primers and agarose gel electrophoresis.

**Results:** Thirty four isolates were confirmed as *K. pneumoniae*. Of the 34 isolates 12 (35%) were susceptible to cotrimoxazole and 20 (59%) to ciprofloxacin. Sixteen (47%) were susceptible to cefotaxime and 21 (62%) to cefepime. Thirty three (97%), 34 (100%) and 33 (97%) of the isolates were susceptible to meropenem, imipenem and ertapenem respectively. Nine (26 %) isolates were producers of extended spectrum beta-lactamases (ESBLs). There was co-resistance to cotrimoxazole and ciprofloxacin in 9 (100%) and 7 (78%) of the ESBL isolates respectively. Genotypically, 12 isolates (35%) had all three genes bla*TEM*, bla*SHV* and bla*CTX-M*. Sixteen (47%) isolates had two gene combinations and 6 (18%) had a single gene group. In 7 (21%) of the 9 isolates that had an ESBL phenotype, all
three genes were amplified.

**Conclusion:** A high (26%) prevalence of ESBL producing *K. pneumoniae* with common beta lactamase gene groups was isolated at Dr George Mukhari Hospital. The emergence of resistance to carbapenems was also observed. The increase in resistance to antimicrobial agents is disquieting as it increases costs of managing patients.

50. Seroprevalence of cytomegalovirus, herpes simplex viruses and rubella virus in women of childbearing age from northern Pretoria

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**Background:** Human cytomegalovirus (CMV), herpes simplex viruses (HSV) and rubella virus infections during prenatal period may lead to pregnancy complications of the mother, abortion, stillbirth, congenital abnormalities at birth, and late presentations of congenital defects in infants. Those virus infections contribute in mortality and morbidity of newborn and under five year old children. We assess the seroprevalence of three virus infections in women of child bearing age from northern Pretoria region serves by Dr George Mukhari hospital.

**Method:** Serological results of women between age 13-45 years old, who were tested for CMV, HSV and Rubella infections between May 2009 and June 2011 were analysed by using MS Excel as a pilot study. Data were obtained from the Prenatal Infections Surveillance Programme of the department of virology, northern branch, NHLS.

**Results:** Seroprevalence rate of CMV-IgG was 100% (529/529) while 19.28% of patients (102/529) were CMV IgM positive. HSV IgG seroprevalence rate was 98.3% (466/474) and 20.4% (93/457) showed positive result for IgM. Rubella antibody level more than 10 international unit/ml was detected in 91.18 % (393/431) and 8.82% (38/431) of women did not have immunity for rubella. Positive rubella IgM was recorded in 0.7% (3/431).

**Conclusion:** Before the age of 46, 100% and 98.3% of women were already exposed to CMV and HSV respectively. CMV exposed pregnant women have a risk of active infection due to reactivation. Although HSV exposed pregnant women may have partial immunity to reinfection, reactivation state can transmit the virus. Rubella immunity was lacking in 8.82% of study population which may acquire primary prenatal infection and posing a threat to unborn child.

51. The usefulness of MTB ACE polymerase chain reaction in detection of *Mycobacterium tuberculosis* from paediatric specimens

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**Background:** TB is estimated to cause the death of more than 450 000 children each year. In children, acid fast bacilli (AFB) results may be negative, culture requires several weeks and other new molecular techniques may take several hours to complete. Therefore there is a need for better, other rapid methods for detection of *Mycobacterium tuberculosis* infection for timeous control measures. This study was done to determine the usefulness of MTB ACE polymerase chain reaction (PCR) in comparison with conventional methods in detecting *M. tuberculosis* from specimens of children suspected of having tuberculosis (TB).

**Method:** One hundred and six specimens, consisting of 17 (16%) gastric aspirates and 84 (84%) sputum specimens were included in the study. These were from children investigated for TB at Dr George Mukhari Tertiary Laboratory, with ages ranging from 2 months to 12 years. Ziehl-Neelsen staining and culture on MGIT were performed. Cultures were confirmed to be *M. tuberculosis* by using TB Ag MPT64 Rapid test. DNA was extracted from specimens and PCR was performed using the Seegplex MTB ACE Detection kit according to manufacturer’s instructions (Seegene Inc, Korea). Sensitivities and specificities of PCR and AFB against culture were determined.

**Results:** MTB ACE PCR detected *M. tuberculosis* in 3 (75%) of 4 culture positive specimens, and AFB detected 2 (50%). The sensitivity of MTB ACE PCR was 75% and specificity 94%. The sensitivity of AFB was 50% and specificity 100%.

**Conclusion:** The specificity of MTB ACE PCR (94%) suggests that this technique may be used to rule out TB. However, the lower sensitivity of this PCR (75%) suggests that this technique may not be best for assessing childhood tuberculosis when urgent diagnosis is needed. These results confirm previous reports that PCR cannot be used alone to confirm TB.

52. Validating the use of rifampicin resistance as a surrogate for multidrug resistance

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**Background:** With the advent of HIV, tuberculosis remains a major health issue, with KwaZulu-Natal (KZN) estimated to have the highest rates of tuberculosis (TB) in South Africa. Dual epidemics of HIV infection and multidrug resistant (MDR) 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.

**Method:** This was a retrospective analysis of all positive TB cases with known susceptibility patterns during 18 months from January 2009 to June 2010.

**Results:** During the five quarters analysed, the percentage of patients with non-MDR, rifampicin-resistant TB ranged from 7.6% in the three quarters of 2009 to 15.6% in the first two quarters of 2010.

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

53. Drug susceptibility testing for third-line anti-tuberculous drugs

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Background: Due to the continually escalating number of cases of multidrug-resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB), there is urgent need for research on newer drugs with different modes of action. Linezolid and clavulanic acid in combination with meropenem (M&C) are currently some of the potential choices. Another area of interest is the search for a faster, more sensitive drug susceptibility test (DST). This helps evaluate the efficiency of TB control programmes and aid in developing strategies to manage the problem of drug resistance. Aims were to validate the agar based DST, by determining its reproducibility, and to evaluate potential third-line anti-TB drugs.

Method: Twelve isolates of varying susceptibilities i.e. sensitive, isoniazid monoresistant, MDR, pre-XDR, and XDR TB were used. They were tested against linezolid, para amino salicylic acid (PAS), capreomycin, and M&C, using the MTT assay and agar dilution method. Isoniazid was the control drug, and H37Rv the control susceptible strain.

Results: MICs obtained by both methods, were similar for all drugs, except MERC. It is vital to note that three of the 4 potential drugs tested revealed exceptional results. Their MICs were low for the MDR, XDR and susceptible isolates i.e. linezolid (0.25-0.5ug/mL), PAS (2-4ug/mL) and M&C (0.5-1ug/mL). The time kill curves revealed similar results, however the trends of isolates of similar susceptibility, were not identical or at the same rate, over the time points.

Conclusion: Linezolid, PAS and M&C are excellent potential third line anti TB drugs. Further work is required to determine possible side effects etc before being administered. The agar dilution method, remains the gold standard due to its high reproducibility. The MTT assay, however is difficult to interpret as it is subjective. An advantage is it is quick, easy and can be used to minimize a range of concentrations.

54. Cytotoxic effects and safety profiles of extracts of active medicinal plants from the Eastern Cape, South Africa

Background: South Africa, medicinal plants, extracts cytotoxic activity, antibacterial activity, herbs, antimicrobial compounds, and respiratory tract infections. Plant-derived antimicrobial compounds that have no or minimal toxicity to host cells are considered candidates for developing new antimicrobial drugs. Safety is therefore critical in formulation of antimicrobials. The aim of this study was to investigate the cytotoxic effects of some South African medicinal plant extracts.

Method: The methanolic and aqueous extracts of nine South African medicinal plants were screened for cytotoxic activities against MAGI CC5+ cells using MTT assay.

Results: The nine plant extracts used in the MTT assay revealed herb 2 (Cyanthula inculta) as the most potent extract identified with activity of (1.4 Cc50 values of 25.6 mg/ml) and induced over 50% of cell death, followed by herb 3 (Croton gratissimus) and herb 4 (Cassine travesaenalis) with activity of (0.2 Cc50 values of 3.7 mg/ml) each. The herbs that induced the least cell death, were herbs 5 (Capris tomentosa) and 7 (Hypoxis hemerocalleida), with the activity of (0.05 Cc50 values of 0.9 mg/ml) each. Of the nine plant extracts 2(22%) exhibited minimal toxicity on MAGi cells and 7(77.8%) exhibited 50% toxicity. In a similar study 2(22%) of the methanolic extracts exhibited anti-HIV1 IIIB activities and against Mycobacterium tuberculosis (TB) only one medicinal plant extract (Lysium inerme) exhibited 29% activity.

Conclusion: In this study, a systematic evaluation of cytotoxic activities of methanolic extracts made from tested medicinal plants showed minimal toxicity on cell lines. Therefore, could be used as natural antimicrobial therapeutic agents for the treatment of respiratory tract infections caused by Haemophilus influenzae and Streptococcus pneumoniae prevalent in Mthatha district, Eastern Cape, with no or minimal toxicity on the host cells.

55. Association between a group of genetically related methicillin-resistant Staphylococcus aureus isolates and maternity and neonatal services in three hospitals in the Western Cape, South Africa, from January 2007 to December 2008.

Background: A previous molecular epidemiology study conducted between 2007 and 2008 identified six distinct groups (designated A – F) of Methicillin-Resistant Staphylococcus aureus (MRSA) isolates circulating among patients in three hospitals in Cape Town. Group C isolates were predominant among patients admitted at Mowbray Maternity Hospital (MMH), but also occurred in patients at the other 2 hospitals, Groote Schuur Hospital and Red Cross Hospital.

Method: We conducted a cohort study including MRSA isolates from all three hospitals to determine whether the group C isolates were associated with prior contact with MMH or the use of maternity/neonatal services. Data on the use of services and admissions were obtained from electronic patient records.

Results: There were 35 Group C MRSA isolates and 54 MRSA isolates of other groups. Group C isolates were predominant among patients admitted to Mowbray Maternity Hospital (MMH), but also occurred in patients at the other 2 hospitals, Groote Schuur Hospital and Red Cross Hospital.

Conclusion: This analysis supports the hypothesis that use of maternity/neonatal services is a risk factor for the acquisition of Group C MRSA. Further studies are planned to determine risk factors for acquisition of MRSA at MMH.
56. Evaluation of the VITEK® 2 anaerobic and Corynebacterium card for identification of anaerobic bacteria

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**Background:** Anaerobes are saprophytic in the human body and are part of normal flora. Under certain conditions, however, they may invade and produce severe infections. The VITEK® 2 Anaerobic and Corynebacterium identification card (ANC) is intended for use with VITEK® 2 systems for the automated identification of most clinically significant anaerobic organisms and Corynebacterium species and does not require anaerobic incubation conditions. It has the advantage of being easy to use, less labour intensive and provides rapid identification compared to the Finegold method.

**Method:** Isolates that were previously tested using the Finegold method and stored in Robertsons cooked meat medium, were subcultured onto 10% Blood agar and incubated for 24/48 hrs anaerobically. Selected isolated colonies were then put up on Vitek panels for identification following manufacturer’s instructions, using a homogenous organism suspension with a density equivalent to a McFarland 3 standard.

**Results:** A total number of 115 isolates (79 gram negative and 36 gram positive) were tested with the Vitek® 2 and compared with the previous Finegold identification results. Of the 79 gram negative isolates, the Vitek® 2 identified 91.13% (72) correctly to genus level and 64.5% (51) correctly to species level, 6 isolates were misidentified and only 1 isolate unidentified. Of the 39 gram positive isolates 89% (32) were correctly identified to genus level and 56% (20) to species level, 3 isolates were unidentified and 1 isolate misidentified.

**Conclusion:** This study indicates that the Vitek® 2 ANC system holds promise as yet another simple, rapid, and satisfactory method for the identification of anaerobes in a clinical microbiology laboratory.

57. Patients with invasive candidiasis, at a referral tertiary hospital in Durban, spectrum and antifungal susceptibility of Candida species isolated from sterile sites

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**Background:** Despite the introduction of new antifungal agents, morbidity and mortality rates associated with invasive candidiasis remain high. Recent data indicate that Candida species are now the fourth most common cause of nosocomial blood stream infections. With the emergence of increasing resistance to antifungals, appropriate empirical therapy for invasive candidiasis is of critical importance. Microbiology laboratories need to provide accurate data on the spectrum, as well as antifungal susceptibility, of Candida species causing invasive candidiasis.

**Method:** 243 Candida species isolated from sterile sites, during January 2010 to June 2011, in the Microbiology Laboratory were analysed. The Candida species were identified by the API ID 32 C (BioMérieux). Antifungal susceptibilities were determined against amphotericin B, fluconazole and voriconazole by ETest (AB Biodisk) in accordance with the manufacturer’s instructions.

**Results:** The most common species identified were C. parapsilosis (128), C. albicans (46), C. tropicalis (28) and C. glabrata (21). With the exception of one C. sake isolate which was resistant to amphotericin B, which also showed decreased susceptibility to fluconazole and voriconazole, the remaining isolates were all susceptible to amphotericin B and voriconazole. Five non-C. albicans isolates were resistant to fluconazole (> 64 ug/ml ), comprising three C. krusei, one C. glabrata and one C. parapsilosis. Eleven other isolates showed decreased susceptibility to fluconazole.

**Conclusion:** C. parapsilosis was the most common species isolated from sterile sites. All C. albicans isolates were susceptible to amphotericin B, fluconazole and voriconazole. A significant number of non-C. albicans isolates showed decreased susceptibility or resistance to fluconazole.

58. The effect of Dodonaea viscosa var. angustifolia (L.f.) on the ultrastructure of Candida albicans cell wall and biofilm formation

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**Background:** Oral candidiasis is an infection that commonly occurs in immunocompromised individuals. The main causative agent is Candida albicans. Many antifungal agents are available and are effectively used. However, due to the development of drug resistance, medicinal plants have been investigated. Dodonaea angustifolia, an indigenous South African plant has an antifungal effect. The crude plant extract also inhibits the adherence of C. albicans to oral epithelial cells, which is the crucial first step of infection. This study investigated the effect of the crude extract on the ultrastructure of C. albicans cell wall, which might be responsible for the inhibition of adherence to oral epithelial cells.

**Method:** Isolates of C. albicans were exposed to 3.125, 1.56 and 0.78 mg/ml crude extract of D. angustifolia and water. Cells were then washed, fixed in glutaraldehyde, postfixed in osmium tetroxide, dehydrated in a series of ethanol washes and embedded in Spurr resin. Ultrathin sections were cut using a glass knife, stained with uranyl acetate and lead citrate, and viewed under the transmission electron microscope at 15 000X magnification. Thickness of cell wall and undulation in cell membrane were graded for 50 cells. Test results were compared to the controls using a pairwise comparison with t-test, ANOVA and a Median test to determine the effect of the plant extract.

**Results:** The crude plant extract made the cell wall of C. albicans significantly thinner compared to the control. The thickness of the cell wall decreased with a decrease in concentrations. The cell membrane was significantly damaged by the plant extract. However, the damage was not dependant on the concentrations.
**Conclusion:** *D. angustifolia* causes structural changes in *C. albicans* which might be responsible for the inhibition of adherence to oral epithelial cells. The actual chemical changes in the cell wall and the active ingredient responsible for these changes need further research.

59. Antimicrobial profile of non-ESBL-producing urinary *Escherichia coli* isolates at DGM Laboratory, Pretoria

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**Background:** *Escherichia coli*, the common causative agents of urinary tract infections have developed resistance to the commonly used antimicrobial agents namely cotrimoxazole and ampicillin. Of concern is resistance even to cephalosporins which is increasing worldwide. Understanding the mechanisms of resistance will assist with planning for appropriate intervention and curb further spread of the problem. This study reports on the antimicrobial susceptibility profile of non-ESBL producing urinary *Escherichia coli* isolates at Dr George Mukhari (DGM) laboratory and the possible mechanisms of resistance to cefuroxime.

**Method:** Non ESBL producing *E.coli* isolates were collected over a period of six months from the National Health Laboratory Services (NHLS) Microbiology department. Susceptibility profile of the isolates was performed according to CLSI standards. PCR for PCR) for the detection of plasmid CMY genes and chromosomal ampC genes was performed on isolates with reduced susceptibility to cefuroxime and/or cefoxitin to assess AmpC hyperproduction as the possible mechanism.

**Results:** Seventy nine percent of the 134 *E.coli* isolates collected were resistant to cotrimoxazole. Resistance to other agents was co-amoxycavulanic acid (13%), ciprofloxacin (6%), cefoxitin (7%) and cefuroxime (4.5%) and nitrofurantoin (2.5%). PCR on 8 isolates showed CMY genes in 4, chromosomal amp C genes in 4, both genes in 3 and 3 isolates had none. There was increased resistance to cotrimoxazole (79%) vs. 72% showed in the same setting in 2006/7. Ciprofloxacin and cefuroxime resistance in non ESBL isolates is of concern. In 3/6 (50%) of the cefuroxime resistant isolates, AmpC beta lactamase hyperproduction is a highly possible mechanism of resistance.

**Conclusion:** Monitoring of resistance to antimicrobial agents need to be monitored continuously. These are preliminary results, the study is on-going and other possible mechanisms of resistance will be studied.

60. Improving Infection control through hand hygiene audit in Tygerberg Hospital in South Africa

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**Background:** Awareness on good hand hygiene practice was identified as one of the focus areas to prevent infection in Tygerberg hospital. The Infection Control Assessment Tool (ICAT) has been used across several countries to monitor and improve infection control practices. The Tygerberg hospital conducted a pilot hand hygiene audits to identify current practices and make recommendations for improvements.

**Method:** Checklists were developed based on the ICAT modules. The audit involved two teams trained in the assessment of the availability of equipment and supplies recommended for good hand hygiene practices and observation of hand hygiene practices in the clinical area. Team members observed the number of patient contacts, the type of health care worker that had contact with the patient, type of contact (invasive/non-invasive), type of hand hygiene prior and after patient contact e.g. hand washing, alcohol rub or none. Audits were conducted on random days and without informing staff before-hand. Audits were conducted in nine wards of the hospital.

**Results:** Most of the recommended infection control practices are followed consistently and thoroughly particularly at units where critical care patients are managed. Infection control practices at the recovery room and labour were however suboptimal. Wearing gloves was identified at the preferred practice instead of washing hands prior to patient contact or contaminated surfaces. Also, it appears normal practice to top up or refill hand soap containers without cleaning the containers first.

**Conclusion:** Hand hygiene audits can indicate health facilities infection control practices. Such audits can generate useful recommendation for reducing infection control including the use of multi-disciplinary team approach to formalise improvement plans.

61. The incidence of upper respiratory tract infections and influenza-like illness among South African Hajj pilgrims in 2010

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**Background:** Upper respiratory tract infection (URTI) is the commonest medical presentation at the Hajj. Prior and subsequent to 2009, influenza vaccination was recommended, but was mandatory for 2009 during the H1N1 pandemic. The H1N1 strain was not present in the southern hemisphere vaccine for 2009, but was in 2010. Our objective was to compare the proportion of URTI in a group of South African (SA) pilgrims attending the 2010 Hajj with previous years, and the proportion of influenza-like illness (ILI) in 2009 and 2010 bearing in mind the change in influenza vaccine recommendations.

**Method:** The principal investigator accompanied a group of 800 SA pilgrims. There were 999 consultations between 26th October & 19th November 2010. URTI was recorded when coryza, pharyngitis, and other ENT conditions were reported. ILI, as per 2009 CDC and Saudi pandemic case definition, was defined as pyrexia of >38.0°C PLUS sore throat and/or cough.

**Results:** URTI accounted for 54.9% (2005), 61.0% (2006), 52.4% (2008) 45.2% (2009) and 46.8% (2010) of presentations for the respective years. Of the 735 pilgrims who...
responded, 318 (43.3%) received influenza vaccine in 2010. The remaining 417 (56.7%) cited unavailability of influenza vaccine. There were 79/735 cases of ILI (10.7%) in 2010, 54/417 (12.9%) in the unvaccinated group and 25/318 (7.9%) in the vaccinated group. Attributable risk of contracting ILI if unvaccinated was 39.3%. Prevalence ratio for contracting ILI if unvaccinated was 1.65 (95% CI 1.05-2.59, p=0.03). There were 43/1085 (3.9%) ILI cases in 2009.

**Conclusion:** URTI proportions did not differ significantly between 2009 and 2010 in South African Hajj pilgrims, yet the proportion of ILI cases was significantly higher in 2010 when the influenza vaccine was not mandatory. Vaccination seemed to confer some benefit against ILI in 2010. A limitation of the study was that pathogens were not laboratory identified.

62. Monitoring antimicrobial resistance of *Klebsiella pneumoniae* at sentinel sites in South Africa


**Background:** *Klebsiella pneumoniae* (KP) is an important nosocomial pathogen and amongst Gram-negative bacteria, the number one producer of extended-spectrum beta-lactamases (ESBLs). We aimed to determine prevalence of ESBL-producing strains and to establish antibiotic susceptibility profiles on significant KP isolates over a one year period.

**Method:** The Antimicrobial Resistance Reference Unit (AMRRU) at NICD initiated active laboratory-based antimicrobial resistance surveillance for nosocomial pathogens from patients with bacteraemia in July 2010. All isolates from 7 sentinel sites were checked for viability and purity, and identification was confirmed using the Vitek 2. Susceptibility testing was performed on MICROSCAN using NM37 panels for *Klebsiella* species.

**Results:** A total of 1541 isolates was recorded as part of established surveillance for nosocomial pathogens. On 925 viable isolates susceptibility testing was performed and 56% presented with ESBL pattern. The in vitro activity was demonstrated as MIC<sub>50</sub> and MIC<sub>90</sub> values respectively, for following agents: amikacin (≤8 and 16, both in sensitive range), gentamicin and tobramycin (≥8 for both, in resistant range), ciprofloxacin (≤0.5 [sensitive] and >2 [resistant]), levofloxacin (≤1 [sensitive] and >4 [resistant]), piperaclillin/tazobactam (≤8 [sensitive] and > 64 [resistant]), ertapenem (≤0.5 for both, in sensitive range), imipenem (≤2 for both, in sensitive range), meropenem (≤1 for both, in sensitive range), tigecycline (≤1 for both, in sensitive range). For penicillins and cephalosporins both MICs values were > 16 and in resistant range.

**Conclusion:** There is high percentage of ESBL KP in our study. MIC<sub>50</sub> indicates that fluoroquinolones and aminoglicoside agents are less active, as are as piperaclillin/tazobactam combination. The highest activity was illustrated by carbapenems and tigecycline. The benefits of this surveillance are in providing evidence for treatment recommendations and the ability to alert clinicians about any new or emerging mechanisms of resistance.

63. An outbreak of hand, foot and mouth disease at Lime Acres in the Northern Cape province of South Africa, 2010

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**Background:** Hand, Foot and Mouth Disease (HFMD), is a common childhood viral infection, caused by an enterovirus. We describe an outbreak of HFMD from 7-19 March 2010, at Lime Acres mine village.

**Method:** We reviewed local clinic and hospital records of suspected HFMD cases. Interviews were conducted with either the patient or their parents (in the case of children). Four throat swab specimens taken from four patients were sent to the National Institute for Communicable Diseases, for virological investigation.

**Results:** We identified 39 suspected cases of HFMD, aged 15 months to 37 years, median 9 years. Twenty (51.3%) were females. Twenty-six (66.7%) were children aged 10 years and below. Thirty-four (87.2%) came from schools and day care centres in the area. Lime Acres primary school was most affected, accounting for 25 (64.1%) of all cases. The six most common symptoms reported were: fever (82.4%), hand lesions (79.4%), sore throat (76.5%), poor appetite (76.5%), foot lesions (61.8%) and headache (55.9%). Laboratory results are still pending.

**Conclusion:** Daycare centre and primary school children were most affected in this outbreak. Most cases were aged 10 years or younger. This finding is consistent with other findings elsewhere (e.g., Goh et al., 1982), where the majority of HFMD cases were found to be aged 10 years or younger. The transmission is from person to person. The concentration of susceptible children in classrooms and the sharing of toys and other teaching tools, are some of the factors enabling the spread of HFMD. We gave health education aimed at affected individuals, their families and learning centres, focusing on information about the disease, its mode of transmission, and the importance of personal hygiene. We also recommended that infected children be kept away from schools or day care centres for at least 10 days after onset of symptoms.

64. Rapid detection of XDR-TB: comparison of the genotype MTBDRSL assay with indirect second-line susceptibility testing

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**Background:** XDR-TB is a serious global health concern. Conventional indirect susceptibility testing for second-line drugs (ofloxacin, kanamycin) is constrained by the relatively slow growth of *Mycobacterium tuberculosis*. Reference
The identification of rapid diagnosis of XDR-TB in cultures. The Genotype MTBDRsl assay allows for the ml on the second-line susceptibility agar. A mutation in codon 91 (S91P) was expressed phenotypically with a growth of 2ug/ml for a fluoroquinolone. Three of the 60 TB isolates were found to be fully susceptible by the indirect susceptibility testing. MTBDRsl assay detected no mutations in drug target genes that code for fluoroquinolone, aminoglycoside and ethambutol resistance. The Genotype MTBDRsl assay was evaluated for its performance and to avoid the transmission of resistant strains. The Genotype MTBDRsl assay was independently evaluated (ie researchers were blinded to the type of strain) on presumptively identified cultures according to the assay methodology. A total of 264 isolates were analysed. Of this 248 and 16 were identified by the presumptive tests as MTBC and NTM respectively. The TB Ag MPT64 Rapid assay identified 248 (94%) of the 264 isolates as MTBC and 16 were found to be inconclusive. The concordance between the TB Ag MPT64 Rapid assay and the presumptive tests was found to be 94%

Conclusion: The TB Ag MPT64 Rapid assay allows for a rapid discrimination between the M.tuberculosis complex and NTM.

66. Rapid detection of bacterial enteric stool pathogens: comparison of the enteric biosystem multiplex PCR assay with routine culture

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Background: In developing countries infectious gastroenteritis is a leading cause of morbidity and mortality. Conventional culture methods remain the gold standard for the isolation of bacterial enteric pathogens in clinical laboratories. However this method is less sensitive for the isolation of Campylobacter and also results in delays in obtaining a culture result. Molecular methods reduce the time to detection and can allow for earlier epidemiological investigations and infection control interventions. An evaluation of the Enteric Biosystem multiplex PCR assay was performed for the simultaneous detection of Campylobacter spp., Salmonella enterica, Shigella spp., and Escherichia coli 0157 from stool samples.

Method: The Enteric Biosystem multiplex PCR assay was performed on approximately 150 stool samples collected from patients with symptoms of gastroenteritis. The overall positivity rate was 6% for routine culture and 8% for the Enteric Biosystem PCR assay. Of the 150 stool specimens processed, the routine culture and Enteric Biosystem PCR assay yielded 94% and 92% negative results respectively. The sensitivity and specificity of the Enteric Biosystem PCR assay was found to be 99% and 98% respectively. Turnaround times for the Enteric Biosystem assay was reduced with results available within 24 hours after receipt of the specimen

Conclusion: The Enteric Biosystem PCR assay is more sensitive and rapid than conventional culture methods for the isolation of bacterial enteric stool pathogens

67. An evaluation of the Genotype MTBDRs+ Assay for the rapid and accurate detection of multidrug-resistant strains of Mycobacterium tuberculosis in extrapulmonary specimens received from patients in high TB burden areas within KwaZulu-Natal

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Background: The HIV/AIDS-MTB syndemic has led to increased extrapulmonary manifestations of tuberculosis. In laboratories that offer second-line drug testing are currently struggling to cope with the increased work-load. Rapid molecular methods to detect drug resistance to second line drugs are necessary to optimize anti-tuberculosis treatment and to avoid the transmission of resistant strains. The Genotype MTBDRsl assay was evaluated for its performance for the rapid detection of XDR-MTB in MGIT cultures

Method: The Genotype MTBDRsl assay was performed on approximately 60 MGIT cultures. Indirect second-line susceptibility testing was performed on all 60 MGIT cultures using Middelbrook 7H11 agar.

Results: The Genotype MTBDRsl identified XDR-MTB in 41 of the 60 MGIT cultures. In 41 isolates the, Genotype MTBDRsl assay identified a single mutation (A90V) in the gyrase A subunit coding for fluoroquinolone resistance, a mutation in nucleic acid postion 1401 (A1401G) of the rrs gene coding for an aminoglycoside/cyclic peptide resistance and a mutation in codon 306 of the embB gene coding for ethambutol resistance.

The second-line indirect susceptibility testing confirmed XDR in all 41 MTB isolates with growths at 2 ug/ml for ofloxacin, 5ug/ml for kanamycin and 7.5ug/ml for ethambutol. The Genotype MTBDRsl assay was evaluated (ie researchers were blinded to the type of strain) on presumptively identified cultures according to the assay methodology.

Results: A total of 264 isolates were analysed. Of this 248 and 16 were identified by the presumptive tests as MTBC and NTM respectively. The TB Ag MPT64 Rapid assay identified 248 (94%) of the 264 isolates as MTBC and 16 were found to be inconclusive. The concordance between the TB Ag MPT64 Rapid assay and the presumptive tests was found to be 94%.

Conclusion: The TB Ag MPT64 Rapid assay allows for a rapid discrimination between the M.tuberculosis complex and NTM.
2009 there were 53411 new cases of extrapulmonary disease in South Africa. High rates of morbidity and mortality are associated with extrapulmonary tuberculosis emphasizing the need for prompt diagnosis and treatment. Diagnosis is hindered by the poor sensitivity of microscopy and the delays in obtaining a culture result. For smear positive sputum specimens, the Genotype MTBDR™ assay has shown to have a sensitivity and specificity of 98.9% and 99.0% respectively for MDR-TB compared with conventional results. To date little data is available on the performance of this assay on extrapulmonary samples.

**Method:** The Genotype MTBDR™ assay was performed on 98 samples comprising 22 smear positive (17 culture positive and 5 culture negative) and 76 smear negative but culture positive extrapulmonary samples, consisting of fluids from sterile sites and lymph node aspirates. Results were compared with the MPT64 antigen assay for MTB identification and conventional and lymph node aspirates. Results were compared with the extrapulmonary samples, consisting of fluids from sterile sites and lymph node aspirates.

**Results:** The Genotype MTBDR™ assay identified MTB with resistance patterns to both drugs in 21 of the 22 (95%) smear positive extrapulmonary samples. The MPT64 antigen assay confirmed the presence of MTB in 21 of the 22 cultures. Of the 76 samples that were smear negative, 26 were culture positive and 50 were culture negative. The Genotype MTBDR™ assay identified MTB with resistance patterns to both drugs in 19 of the 26 (73%) smear negative, culture positive samples. The MPT64 antigen assay confirmed the presence of MTB in all 19 cultures. The Genotype MTBDR™ assay has a sensitivity of 95% for the detection of MTB in smear positive fluid aspirates and 73% for smear negative fluid aspirates.

**Conclusion:** The Genotype MTBDR™ has shown to be useful for the diagnosis of MDR-TB in extrapulmonary fluid aspirates.

68. Development of a genotypic test for the detection of KwaZulu-Natal strain of *Mycobacterium tuberculosis*

**Background:** The F15/LAM4/KZN strain has been the cause of a considerable number of drug-resistant tuberculosis cases in KwaZulu-Natal. Most studies to date have focussed on drug-resistant isolates. However, the distribution of this strain in KZN is unknown, and there are no data from an epidemiologically defined population that includes drug susceptible isolates. To assess the prevalence and distribution of the F15/LAM4/KZN strain within the province, a strain specific molecular test was developed and used to screen isolates collected as part of a province wide drug susceptibility survey.

**Method:** Deletion analysis has been standardised for six major lineages of *M. tuberculosis*, including the Beijing family and in this study, it was developed for the F15/LAM4/KZN strain. A range of large sequence polymorphisms (LSPs) were identified by comparing publically available genome sequences which were specific for the F15/LAM4/KZN strain. Two LSPs were chosen and validated as strain specific markers using 112 clinical isolates of known RFLP and spoligotypes. A multiplex Real Time PCR assay was developed to detect an LSP characteristic to the KZN strain (fadE22), in conjunction with a probe for the RD105 deletion, previously described for the Beijing strain, screening for the KZN and Beijing strains was carried out on 777 isolates collected from multiple sites in KwaZulu-Natal. The Pearson chi square test was used, cross-tabulating genotype to site and genotype to drug resistance.

**Results:** This study represents the first effort to demonstrate the use of LSP analysis in KwaZulu-Natal. A key finding was that the KZN strain was not the predominant strain in KZN, making up 5.1 and 5.4% of the inpatient and outpatient groups respectively. The Beijing strain accounted for 18% and 29.8% of the inpatient and outpatient groups respectively. The KZN strain was more likely to be associated with drug resistance than the Beijing strain in both the inpatient and outpatient groups.

**Conclusion:** These findings demonstrate the usefulness of LSP analysis as a screening tool and indicate its pertinence to epidemiological investigations. More studies focused on screening of clustered strains could reveal transmission dynamics within a population and highlight the characteristics of cluster strains within the geographical spread.

69. Invasive group B streptococcal disease in South Africa

**Background:** Group B Streptococcus (GBS) is a leading cause of neonatal sepsis and causes substantial morbidity among adults (especially elderly) in developed countries. Little is known about the burden of GBS in developing countries or areas with high HIV prevalence.

**Method:** We analyzed data on all invasive GBS isolates cultured in 2010 at National Health Laboratory Services (NHLS) public sector laboratories, which serve >80% of the population. We estimated incidence using GBS isolated from cerebrospinal fluid (CSF) and blood cultures (BC), and Statistics SA population and live birth (LB) data. Age-specific incidence was calculated for national and Soweto-specific data. Cases from days of life 0-6 and 7-89 were considered early-onset (EOD) and late-onset disease (LOD) respectively.

**Results:** In total, 615 invasive isolates were reported in 2010 (81.1% from BC and 17.1% from CSF), with 132 (21.5%) from Soweto. Estimated national incidences for EOD and LOD were 0.19 and 0.19/1000 LB respectively; Soweto-specific EOD and LOD were 1.40 and 1.43/1000 LB. Adult incidence was 0.30/100,000 nationally and 2.76/100,000 in Soweto (18-39 yrs at 2.34; 40-64 years at 3.44 and >=65 years at 3.06/100,000).

**Conclusion:** Incidence rates of invasive GBS disease in Soweto were consistent with previously published data on neonatal GBS in Soweto, and 7-9 times higher than national estimates, suggesting under-ascertainment of cases at a national level. The age distribution of cases – similar rates of EOD and LOD and highest adult rates among those aged 40-64 years – contrasts with patterns seen in developed countries. The
reasons for these differences are unclear and warrant further study. Efforts are needed to improve case detection nationally, since data from Soweto suggest a substantial burden of GBS disease in South Africa, and a need for prevention efforts.

70. Causes of meningitis in the era of HIV infection

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Background: Meningitis is a life threatening infection of the central nervous system that has a high morbidity and mortality rate. Knowledge of the commonest etiologies is important to guide empiric treatment. In the 1980s the commonest causes of bacterial meningitis were Neisseria meningitidis, Streptococcus pneumoniae and Haemophilus influenzae. There have been no previous published studies on the causes of meningitis in adult patients at King Edward VIII Hospital, KwaZulu-Natal, a setting with high HIV prevalence.

Method: Data was retrospectively extracted from microbiology laboratory records. Cerebrospinal fluid (CSF) specimens from adult patients from 1 January 2008 to 31 June 2010 were included. Only specimens from which microorganisms were cultured were analyzed. Duplicate specimens with the same organism were excluded. Organism cultured, cell count and demographic characteristics of the patients were noted.

Result: During the 30 month period, 260 patients had one or more culture positive CSF specimens. 47% of the isolates were Cryptococcus neoformans, 24% Mycobacterium tuberculosis, 11% gram negative bacteria, 9% Streptococcus pneumoniae and 9% other. There was an increase in the proportion of C. neoformans cultured over time during the study period, with C. neoformans contributing to 31 % of isolates in 2008, 47% in 2009 and 63% in 2010. Three patients had infection with both C. neoformans and M. tuberculosis. Majority of the patients were in the 21 - 50 age group.

Conclusion: HIV infection has lead to a change in the spectrum of microorganisms causing meningitis, with a dramatic increase in opportunistic pathogens such as C. neoformans. TB culture on CSF specimens is an important laboratory investigation in this setting. C. neoformans and M. tuberculosis are now the commonest causes of culture positive meningitis in King Edward VIII Hospital.

71. Spread of multidrug-resistant tuberculosis, including extensively drug-resistant tuberculosis, in rural KwaZulu Natal

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Background: Tugela Ferry in KwaZulu-Natal South Africa reported the largest cluster of extensively drug-resistant tuberculosis (XDR TB) cases in the world. It is unknown whether a single clone of the drug resistant strain or multiple strains is circulating in this area. This study investigated the molecular epidemiology of Mycobacterium tuberculosis (MTB) in Tugela Ferry, to ascertain whether the cases of multidrug-resistant (MDR) including XDR TB are the result of clonal spread or simultaneous de novo development of resistance in different strains.

Method: MTB isolates from 2005/6 and 2008/9, with varying susceptibility profiles were genotyped. Spoligotyping and IS6110 Restriction Fragment Length Polymorphism (RFLP) were used to establish the circulating genotypes.

Results: 99 isolates were classified into 16 SITs and 2 orphan spoligotypes. Upon further genotyping by IS6110 RFLP 46 different banding patterns were observed. Among XDR TB isolates, SIT60 spoligotype was predominant and all belonged to the F15/LAM4/KZN family by RFLP analysis. The SIT34 was predominant among MDR isolates and these had banding patterns belonging to the F28 family. MDR isolates with the SIT60 spoligotype displayed the original F15/LAM4/KZN IS6110 genotype reported in 1994. Additional spoligotypes and unique RFLP patterns were also present. Among the susceptible isolates various genotypes were present.

Conclusion: In Tugela Ferry the Beijing strain is circulating in a susceptible form although it has been associated with resistance in different geographic areas. Using 2 complementary genotyping methods, we showed that the MDR strains present are the result of clonal spread associated with the F28 family, as well as de novo resistance which manifests as unique patterns. The XDR epidemic in Tugela Ferry is the result of clonal spread and RFLP patterns suggest this with evolving genotypes belonging to the F15/LAM4/KZN family. Spoligotyping alone cannot be used for epidemiological purposes since SITs have been found among isolates belonging to the same IS6110 RFLP family.

72. Is this the first case of proven HIV transmission via surrogate breastfeeding?

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Background: Breastfeeding is a well known mode of transmission of the human immunodeficiency virus (HIV) from mother to child (MTC), with a transmission frequency of more than 16% in some areas. The probability of transmission per liter of ingested breastmilk is similar to that via heterosexual contact (0.0003-0.0015). In 2005 results from a study in South Africa indicated that shared breastfeeding by non-biological caregivers was the single most important factor (other than MTC transmission) linked with HIV infection in children.

Case report: In 2009 a 74-day old infant was hospitalised in respiratory distress. She tested positive for HIV. The infant’s mother was reportedly HIV negative during pregnancy, which was subsequently confirmed. A repeat ELISA on the infant remained positive and a further positive DNA polymerase chain reaction (PCR) proved that the infant was HIV-infected. The mother reported that her sister had breastfed the infant intermittently from six weeks of age. The sister and her own five-month old child were subsequently found to be HIV-
positive by ELISA and DNA PCR respectively. Plasma samples from the sister and both infants were used to perform partial sequencing of the HIV pol gene using the TruGene HIV-1 genotyping kit. Phylogenetic analysis supported the epidemiologic conclusion that the surrogate breastmilk was the source of the infant’s HIV infection.

**Discussion:** To our knowledge this is the first case where phylogenetic evidence supports the epidemiologic link between shared breastfeeding and HIV infection of the recipient infant. Lack of knowledge regarding breastmilk as mode of transmission is rife in many African countries, not only among the general population, but also among health care workers. This case highlights the importance of continued education regarding the risk of HIV transmission via breastmilk.

### 73. An evaluation of a novel culture supplement to improve *Mycobacterium tuberculosis* growth in liquid medium

**Background:** Resuscitation-promoting growth factors are a family of proteins able to restore culturability from a dormant state as well as stimulate the growth of viable bacteria. Rpf-like factors, found in high concentration in the culture filtrate of log-phase cultures, have been shown to enhance growth in *M. tuberculosis*. We hypothesize that culture filtrate may enhance the growth of *M. tuberculosis*(MTB) in liquid culture and may improve time to detection in clinical samples.

**Method:** H37Rv was cultured in 7H9 liquid broth at 37°C to mid log phase. Culture filtrate (CF) was harvested and filtered using a 0.22µ filter. Sterility was confirmed by incubating in mid log phase. Culture filtrate (CF) was harvested and filtered using a 0.22µ filter. Sterility was confirmed by incubating in 

**Results:** There was a marked difference in CFU between supplemented broth and standard broth (difference in CFU for H37Rv = (2.93±1.57) X 10^7, for RXH202 = (2.12±0.2)X10^7 and for RXH379 = (3.13±0.70) X 10^6at 220 hrs.; (Mean±SD). Further, we detected the effect of CF on time to detection (TTD) in MGIT using a range of clinical strains of MTB(MGIT inoculated with 1, 10, 100 or 500 CFU). MGIT supplemented with CF showed considerable reduction in TTD (reduction = 4.70±0.40 days, mean±SD). Presently, we are evaluating whether CF can be used to reduce TTD using clinical sputum samples.

**Conclusion:** Our data suggest that CF has potential to enhance the growth of MTB in liquid medium. Further work will determine whether this finding can be useful in reducing time to detection for clinical samples.

### 47. Trend analysis of proficiency testing results for bacteriology

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**Background:** The Bacteriology Proficiency Testing Scheme is provided by the External Quality Assessment Reference Unit at National Institute for Communicable Diseases. It is conducted every four months and the results are monitored continuously to ensure timely interventions when problems are identified. We aimed to analyze results obtained from participating laboratories over a 27 month period.

**Method:** In 2009, 128 laboratories participated in the Bacteriology Proficiency Testing Scheme survey 1 and 129 laboratories participated in surveys 2 and 3; while 116 laboratories participated in 2010 and 113 in 2011. A survey consists of a set of four simulated samples with the corresponding instructions for laboratory testing including the clinical details. A response form is included in the documentation for participants to complete and return to the provider.

**Results:** Three surveys were completed in 2009 and 2010 respectively and one in 2011. However not all laboratories returned completed responses. We obtained results for each sample depending on what grading category was requested i.e. microscopy, culture and identification, serotyping and antimicrobial susceptibility testing. We analysed the acceptable responses for each category in each survey. The percentage varied over the 27 month period from 31.5% to 99.1%. Since culture and identification is performed on all isolates, the percentage of acceptable results was analysed in order to determine a trend of performance which ranged from 40% to 98%. *Vibrio cholerae* was sent in simulated stool in two of the seven surveys evaluated. One isolate was a serogroup O1 and the other was non O1. In both surveys this isolate was the most poorly identified. However, other organisms such as *Klebsiella pneumoniae* was correctly identified (95.8% and 98.2%).

**Conclusion:** This trend analysis reveals that participating laboratories have problems with identification of organisms isolated from different specimens. This provides an indication that there is a need for improvement and perhaps further training of laboratory staff, as it is essential for accurate and reliable identification and susceptibility testing of microbiological organisms so that the appropriate treatment can be administered.

### 75. Study of monitoring antimicrobial resistance of *Staphylococcus aureus* at sentinel sites in South Africa

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**Background:** *Staphylococcus aureus* exhibits three problematic features that taken together are not found among most other clinically-relevant bacteria. This organism expresses virulence factors and is almost always considered as clinically significant when isolated from sterile sites. Also the organism develops and expands resistance to include a
Conclusion: and 49% were resistant to oxacillin (MRSA). The in vitro susceptibility testing was performed as part of established surveillance for nosocomial pathogens. On 1017 viable isolates, 1266 isolates were recorded during one year. The susceptibility testing was performed on MICROSCAN using PB28 panels for Staphylococcus aureus. We established MIC50 and MIC90 breakpoint values and percentage of susceptibility on significant isolates during period of one year.

Results: Total numbers of isolates were recorded as part of established surveillance for nosocomial pathogens. On 1017 viable isolates, susceptibility testing was performed and 49% were resistant to oxacillin (MRSA). The in vitro activity was demonstrated as MIC50 and MIC90 breakpoint values for following agents: azithromycin ($\leq 2$ [sensitive] and $>4$ [resistant], respectively), erythromycin ($\leq 0.5$ [sensitive] and $>4$ [resistant], respectively), for mupirocin values were sitting at $\leq 32$ for both breakpoints. Vancomycin is exhibiting susceptible activity and MIC breakpoint was 1 for both values, linezolid MIC50 was $\leq 2$ interpreted as sensitive and MIC90 was $>8$ as resistant. For majority tested β-lactam antibiotics MIC50 and MIC90 values were in resistant range.

Conclusion: High percentage of MRSA and MIC90 value in resistant ranges for majority of agents indicates importance of surveillance of susceptibility pattern particularly when exhibit decreased activities against Staphylococcus aureus as indicated in this study.

76. Invasive fungal isolates by risk group at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), 2006-2010

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Background: The spectrum of invasive mycoses amongst certain high risk populations is growing in addition to Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus, emerging opportunistic fungi are increasing in importance.

Method: A search of the CMJAH NHLS microbiology laboratory database was conducted from 2006-2010. Data on patient location by ward and non-CSF sterile site fungal identification and susceptibility, was analysed.

Results: Of 429 fungal isolates, 425 (99%) were yeasts. Candida species contributed to 238/425 (56%) of these, with C. albicans comprising 128/238 (54%). The most common non-albicans Candida species were C.parapsilosis 47/110 (43%) and C. glabrata 19/110 (17%). Non-albicans Candida species predominated in paediatric haematology-oncology 24/33 (73%) and neonatal critical care 29/52 (56%) patients. Cryptococcus neoformans accounted for 182/425 (42%) yeasts, with 174 (96%) originating from adult medical patients. Rhodotorula species, Saccharomyces cerevisiae and Trichosporon asahii, contributed 5/425 (0.9%). Moulds 4/425 (1%) included Mucor species, Aspergillus fumigatus and Chrysosporium species. Fluconazole and voriconazole MICs for C albicans were determined on 97/128 (76%) and 82/128 (64%) respectively. Non-susceptibility was 2/97 (2%) for fluconazole, and 1/82 (1%) for voriconazole. Of the 87/110 (79%) non-albicans Candida species tested against fluconazole, 25/87(29%) were non-susceptible with 6/82 (7%) testing non-susceptible to voriconazole.

Conclusion: Candida species was the most common fungal isolate from non-CSF sterile sites in critical care units. Non-albicans Candida species, rivalled, and in neonatal and paediatric haematology-oncology populations, surpassed, C. albicans as the leading fungal pathogen. Cryptococcus neoformans predominated in non-ICU adult medical patients. A few other opportunistic yeasts and moulds have surfaced in critical care units. The spectrum of invasive fungi, in high risk groups, at this tertiary academic hospital, mirror global epidemiological trends.

77. Emerging carbapenem-resistant Enterobacteriaceae

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Background: Carbapenem-resistant Enterobacteriaceae (CRE) are usually resistant to all β-lactam agents as well as most other classes of antimicrobial agents. CRE cause severe infections among residents of long-term-care facilities. The treatment options for patients infected with CRE are very limited. Tigecycline and polymyxins including colistin have been used with variable success. Healthcare-associated outbreaks of CRE have been reported in other countries. CRE are increasingly recognized as the cause of sporadic and outbreak infections in the U.S. Aggressive infection-control practices are required in aborting these outbreaks. Aim and objectives were to follow the new laboratory methodology for antimicrobial susceptibility testing by checking the zone diameter and MIC breakpoints of carbapenems for Enterobacteriaceae (new 2011 CLSI Guideline) and to record the CRE prevalence and implement the infection control practices.

Method: The prevalence of CRE was calculated manually for 2009 and 2010. Data records were collected from VITEK2® and computer Whonet system.

Results: CRE isolates are also emerged in our local hospital in 2009 and 2010. The prevalence percentage of each CRE isolate will be shown with graph in poster. Detection of carbapenemases and
implementation of infection control practices are necessary to limit spread.

78. Common microorganisms isolated from endotracheal aspirates of adult patients from the intensive care unit of a central referral hospital, KwaZulu-Natal

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Background: Inappropriate initial antimicrobial therapy in patients with nosocomial pneumonia, including health care associated pneumonia (HCAP), is associated with excess mortality. Therefore, it is important to have the common microorganisms most often associated with this infection as records yearly. Aim and objectives were to investigate the prevalence of common microorganisms isolated from ETA and their resistant antibiogram. To approach the proper management of nosocomial pneumonia in ICU Patients based on common significant isolates records yearly.

Method: We analysed the data from computer (Whonet), records of daily ward round over a period of one year and that analysis will be ongoing for unit specific records. The number of each isolate, type of specimens received and percentages of resistant microorganisms/statistics were calculated manually by pathologist.

Results: The total number of different microorganisms were (230) from different types of specimens. (154/230= 67%) were isolated from ETA of ICU patients during the study period 2009. Analyzed each organism from ETA showed antibiotic susceptibility pattern; (41/41=100%) were multi-drug resistant Acinetobacter spp and ESBL positive Klebsiella pneumoniae and E.coli were (N= 8/15) 53% and (N=2/4) respectively. (6/11) 55% of Saureus was MRSA isolated from ETA.

Conclusion: Nosocomial (HCA) pneumonia is predominant among the nosocomial infections in ICU according to study (2009). Acinetobacter spp associated nosocomial pneumonia is predominant and mainly associated with intubations and mechanical ventilation in patients in intensive care units. And followed by ESBL producing K. pneumoniae. E.coli and MRSA were sporadic occurs in the unit. Equipments and hands contaminated with secretion, aerosols or droplets are the main route of transmission of micro-organisms. Due to the MDR Gram negative and Gram positive bacteria were colonized or significance pathogens, infection control practice should be enhanced properly combined with direct antimicrobial therapy.

79. Outbreak of a clone of vancomycin-resistant enterococci in multiple wards at a Johannesburg academic hospital

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Background: Vancomycin-resistant Enterococci (VRE) are important nosocomial pathogens causing considerable morbidity and mortality worldwide. Transmission usually occurs via direct contact with colonised or infected patients, and subsequent horizontal transmission via the hands of health-care workers, contaminated patient-care equipment or environmental surfaces. Another risk factor is the use of broad-spectrum antimicrobials. Outbreaks of VRE are well documented and usually clonal in origin. Over the past 12 months there has been a dramatic increase in the incidence of VRE in academic hospitals in the Johannesburg area

Method: From April 2010 to June 2011, 77 VRE were cultured from patients in multiple wards at a Johannesburg Hospital. These isolates were tested for glycopeptide resistance using the E-test®, genotyped with the HAIN GenoType Enterococcus Assay, and a subset were analysed for clonality by macro-restriction analysis using pulsed-field gel electrophoresis (PFGE).

Results: All 77 isolates were identified as Enterococcus faecium. Sixty nine of these isolates were phenotypically characterized as VanA Enterococcus faecium with high minimum inhibitory concentrations to vancomycin (MIC >25µg/ml) and teicoplanin (MIC >32µg/ml). A further 8 were characterized as VanB Enterococcus faecium associated with high-level vancomycin resistance and susceptibility to teicoplanin. These phenotypic characteristics were genotypically confirmed using the Hain assay. Macro-restriction analysis revealed a major clone (13 isolates) of VRE in the hospital with 2 smaller clusters and 3 unique isolates. One of the smaller clusters (4 isolates) differed from the major clone by a single band, thus making them closely related. The clonal isolates were from patients in various wards separated both by discipline and location.

Conclusion: These findings confirm the spread and evolution of a clone of VRE within the hospital. This highlights the importance of timeous implementation of appropriate infection control interventions to prevent the spread of these organisms.

80. Validation of the Abbot Real-Time High Risk HPV Assay in a Johannesburg institution for detection of high risk HPV in cervical specimens.

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Background: Human papillomavirus (HPV) is estimated to be the most common sexually transmitted infection and a common infection of epithelial tissues. It has previously been shown to be a causative agent in the development of cervical cancer, which is one of the leading causes of death in women in the developing world. There is therefore an urgent need for reliable screening methods for the detection of the high-risk HPV genotypes.

Method: The Abbot Real Time High Risk HPV assay is a qualitative assay that amplifies and detects High Risk HPV (HR HPV) DNA in cervical specimens collected in liquid media. DNA extractions were performed using the Abbot m2000sp instrument. HPV targets are amplified by PCR using five primers targeting a conservative L1 region of HPV. The
Abbot m2000rt instrument is used for the amplification and detection of the HR HPV. For the validation and reliability of this assay: Thirty four (n=34) cervical samples and five (n=5) VQA samples were analysed for the detection of HPV.

**Results:** The high risk HPV DNA genotypes detected in this validation study were HPV16, HPV18 and other HR HPV with a mean cycle number of 22.08, 24.63 and 21.95, respectively. The endogenous Human B-globin was detected in all samples analysed, showing sample validity and cell adequacy for this assay. It was found that 25/39 samples were positive, and 14/39 were negative for HPV. 100% reproducibility was obtained.

**Conclusion:** The High Risk assay is a reliable assay that detects and differentiates the known high risk HPV genotypes. The risk of contamination is limited, as both extractions and amplification are automated. Therefore this assay could be used as a potential screening assay for HPV.

81. Clonal distribution of invasive serogroup 6 pneumococcal isolates from children less than five years of age, South Africa, 2007

**Background:** Streptococcus pneumoniae serogroup 6 (serotypes 6A, 6B, 6C and 6D) cause invasive pneumococcal disease (IPD) predominantly in children and are associated with antimicrobial resistance. In South Africa in 2007, 6B and 6A were the second and third most common serotypes, respectively, in children <5 years old, prior to Background of IPD. Serotypes were determined by Quellung. Multilocus sequence typing (MLST) was performed on 147 systematically selected (every 2nd) isolates for 6A

**Results and conclusion:** 14 kinds of IPD cases were reported, of which 1468/4531 (32%) were among children <5 years of age. Of these, 1085 (74%) had viable isolates and 287 (20%) were identified as serogroup 6. 6B isolates were more likely to be penicillin non-susceptible than 6A isolates (120/147, 82% vs 69/136, 51%; p<0.001). 6C isolates were penicillin susceptible. 6D comprised 27 STs differentiated into four clonal groups [ST2285 (n=14), ST2289 (n=10), ST6305/ST6303 (n=2) and 11 singletons. 6B comprised 34 STs forming four clonal groups [ST185 (n=18), ST4929 (n=17), ST2289 (n=4), ST6305/ST6303 (n=2)] and 11 singletons. 6A and 6B shared STs (ST185, ST1094, ST2285, and ST2289). 6C comprised 2 related STs, ST6310 (n=2) and ST2185 (n=1), and one singleton (ST6311).

**Conclusion:** Serogroup 6 is diverse and whilst some STs have been identified elsewhere, the majority were unique to South Africa. ST185, the South Africa6B-8 global clone, continues to circulate among serotype 6B. Common STs were detected in serotypes 6A and 6B, suggestive of capsular switching. Serotype 6C STs were unique.

82. Nasal carriage of *Staphylococcus aureus* in paediatric outpatients

**Background:** *Staphylococcus aureus*, a major organism in the nasal cavity, colonizes the skin and nares, producing variety of invasive enzymes and toxins, is a common pyogenic bacterium. Wounds through the skin, sweat glands, hair follicles and other body sites, can cause skin - soft tissue infections, systemic infection and respiratory infection.

Despite advances in antimicrobial therapy and intensive care support, *S. aureus* continues to cause significant morbidity and mortality. The objectives were to determine the prevalence of *Staphylococcus aureus* nasal carriage in paediatric outpatients at CH Baragwanath hospital, to determine the presence of PVL and *MecA* in the *S. aureus* isolated, to determine the antibiotic susceptibility profile and their genetic relatedness by ‘Genotype Staphylococcus’, to highlight the importance of early detection of potential community-associated *S. aureus*.

**Method:** A dacron swab was used to swab the nasopharyngeal cavities of 68 patients. The swab was cultured onto blood and bacitracin heated blood agar. Identification and susceptibility testing was be performed by routine methods following Clinical Laboratory Standards Institute guidelines. Bacterial colonies resembling *Staphylococcus aureus* were isolated. Molecular typing using ‘HAIN Lifescience – Genotype Staphylococcus’ was performed to differentiate *Staphylococcus* strains and to detect the presence of the PVL and *MecA* gene.

**Results and conclusion:** 14 strains of *Staphylococcus aureus* were isolated of which 12 strains were tested by the ‘Genotype Staphylococcus’. The study showed that 3 of the 12 strains (25%) were positive carrying the PVL gene and all strains were negative for *MecA* gene. 2 of the 3 patients presented with symptoms of skin rash. The nasal carriage rate of *Staphylococcus aureus* was found to be 21% and no MRSA was isolated. The isolates were generally susceptible to antibiotics except for 13 strains resistant to penicillin and 2 strains intermediate resistant to gentamycin. Screening of potential carriers for significant isolates and their resistant patterns would benefit policy makers in allocating scarce resources efficiently.
83. Second-line drug resistance profiles among death patients of multidrug-resistant tuberculosis treatment in South Africa

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Background: Multidrug-resistant tuberculosis (MDR-TB) occurs through either primary resistance (infection by M. tuberculosis already resistant to isoniazid and rifampicin) or through the selection of drug resistant mutants due to inadequate therapy, poor patient compliance etc. The Medical Research Council (MRC) has since 2000 been commissioned by the National Department of Health to coordinate and monitor the implementation of a standardized approach to the management of MDR-TB in South Africa. This initiative, also referred to as DOTS-Plus for MDR-TB, aims to evaluate the care of MDR-TB patients under a public health approach. The aim was to determine the second-line (SL) drug resistance profile among patients who died (DOTS-Plus study 2000-2004) during their MDR-TB treatment, using the GenoType MTBDRsl line probe assay (Hain LPA).

Method: Patients from all 9 provinces in SA (N=2079) were enrolled into the DOTS-Plus study. From the 2079 MDR TB patients, 367 (18%) died during the treatment period. For the purpose of this study, the last positive confirmed MDR TB isolate (Lowenstein Jensen slant) available from 186 death cases were tested for second line drugs using the GenoType MTBDRsl line probe assay (Hain LPA) for rapid molecular detection of resistance in cultures isolated.

Results: From the 186 cultures tested, results were obtained for 156. Due to the total disintegration or drying of the Lowenstein Jensen slants, 30 strains revealed no bands on the TUB locus indicating the absence of mycobacterium belonging to the Mycobacterium Tuberculosis (MTB) complex. Of the 156, 16.7% (26/156) were found to be extreme drug resistant (XDR) and 13.5% (21/156) pre-XDR (resistant to fluoroquinolones or aminoglycosides). 15 and 6 of the pre-XDR strains showed resistance to fluoroquinolones and aminoglycosides respectively. 48.7% (76/156) were fully susceptible to both the fluoroquinolones and aminoglycosides. 21.2% (33/156) of results were inconclusive for the aminoglycosides due to the absence of all bands on the rrs loci.

Conclusion: The difference in susceptible and resistant rates (49% susceptible vs 30% XDR and pre-XDR) for second line drugs observed in the death cases in this study were insignificant, indicating resistance to fluoroquinolones and/or aminoglycosides not being an indicator for unfavorable outcome. The reason for the deletion of the bands on the rrs loci (aminoglycosides) needs further investigation.

84. Recurrent Group B streptococcal infection in a neonate

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Background: Group B streptococcus (GBS) is an important cause of meningitis and bacteremia in neonates. GBS infections may be classified as early (occurring in the first week of life) due to vertical transmission from the mother or late (from 7 days to 3 months of age) due to horizontal transmission from the mother or other persons. Here we present the case of a neonate with recurrent GBS infection.

Case report: The child was born at 28 weeks’ gestation by normal vaginal delivery to an HIV-positive mother with a CD4 count of 257 cells/mm³. The birth weight was 710g. After birth the baby was treated empirically for presumed sepsis with 5 days of intravenous penicillin and gentamicin. On day 23 of life the child manifested new signs of sepsis and was commenced on penicillin and amikacin. GBS was isolated from blood but not from cerebro-spinal fluid. The child received ten days of penicillin with a good clinical response. Subsequently the child remained in the neonatal unit and appeared well despite occasional convulsions. On day 58 of life the child again appeared septic. GBS was isolated from both blood and CSF and the child responded to treatment with penicillin.

Discussion: This child experienced recurrent culture proven GBS sepsis despite seemingly adequate initial treatment and a good clinical response. It is difficult to determine whether the 2nd episode was due to a relapse of the original infection or to re-infection. While molecular typing will be attempted, this may not be able to provide an answer, as re-infection could occur with the same strain. Possible predisposing factors to recurrent infection in this patient include prematurity, extremely low birth weight and HIV exposure. The literature concerning duration of therapy and risk of recurrent GBS infections will be discussed.

85. The Vaccines for Africa Initiative: promoting evidence-based vaccine advocacy in Africa

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Background: Between 1990 and 2007, under-five mortality rate in Africa reduced by only 20% from 181 to 145 deaths per 1000 live births. Vaccine-preventable diseases are a major contributor to the high child mortality in our continent; as a result of limited vaccine Background and low immunisation coverage. However, effective and sustainable vaccine advocacy can mobilise resources for national Expanded Programmes on Immunisation (EPI), encourage wide participation and local ownership of EPI services, and lead to positive changes in knowledge and attitudes towards vaccines among parents and healthcare providers in every African country. This was the rationale for setting up the Vaccines for Africa Initiative (VACFA) in 2008; to promote vaccine-focused, evidence-based advocacy and communications across Africa. VACFA’s vision is “An Africa free of vaccine-preventable diseases.”

Method and results: We conduct a series of Africa-focused vaccine advocacy, communication and capacity-building activities each year. In 2009 we launched an irregularly updated vaccine advocacy website (www.vacfa.com), which aims
to be a ‘one-stop shop’ of targeted information for health professionals, policymakers, programme managers, and parents. The website serves as an interactive forum for exchange of up-to-date and evidence-based Africa-focused information on vaccines. VACFA also runs the Annual African Vaccinology Course, which is now in its seventh year. More than 400 EPI managers, medical doctors, nurses, public health practitioners, and scientists have attended the course. In addition, VACFA members provide technical support and advice to immunisation practitioners and organisations at all levels. Finally, research within VACFA is focused on systematic reviews and knowledge transfer.

**Conclusion:** VACFA is ideally positioned to make a significant impact to the new Decade of Vaccines’ initiative. We plan to expand our activities by taking advantage of existing infrastructure, expertise and opportunities to contribute to decreasing vaccine-related child mortality in Africa through evidence-based vaccine advocacy and communication.

**86. Building evidence for improving communication about childhood vaccinations in low- and middle-income countries**

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**Background:** Effective provider-parent communication can improve childhood vaccination coverage and strengthen immunization services in low- and middle-income countries (LMICs). However, demand-side interventions to improve vaccination coverage have been neglected and existing rigorous research is often not readily found or easily applicable to LMICs. This makes it difficult for policy makers to use evidence to inform policies and practice. To describe approaches used by the ‘communicate to vaccinate’ (COMMVAC) project to explore, evaluate and disseminate evidence on strategies for improving communication about childhood vaccinations with parents and communities in LMICs.

**Method and results:** COMMVAC uses a combination of methods. First, we produced a systematic map of communication interventions. Systematic maps use the same rigorous methods as systematic reviews of effects but focus on describing the range of interventions. For each intervention identified, we extracted information on the population(s) targeted, settings, and intervention purpose and delivery (and evaluation design and outcomes in trials). Second, we developed a taxonomy of interventions to improve communication around childhood vaccination so as to: (1) understand the relations between different types of interventions; (2) facilitate conceptual mapping of these interventions; and (3) clarify the key purposes of interventions. Third, we will hold deliberative fora with key stakeholders to discuss priorities for systematic reviews of effects, informed by the systematic map and taxonomy. Fourth, we will conduct systematic reviews on high priority topics. Finally, we will produce web-based evidence summaries that translate the review findings into accessible messages for LMICs and allow users to add implementation commentaries.

**Conclusion:** COMMVAC takes a novel approach to building knowledge resources and making more effective use of existing research and practice descriptions. Key outputs will include high quality evidence on the scope and effects of interventions to improve provider-parent communication around vaccination and knowledge resources tailored for LMICs.

**87. Vaginal microbicides for prevention of HIV and other sexually transmitted infections: systematic review and meta-analysis**

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**Background:** Two decades of research on vaginal microbicides for prevention of HIV infection have had limited success. However, new randomised controlled trial (RCT) data have recently been published; but these have not yet been the subject of a systematic review.

**Method:** In June 2011 we conducted an exhaustive search for RCTs that have assessed the effects of vaginal microbicides for prevention of HIV and sexually transmitted infections (STIs). We assessed study eligibility and methodological quality, extracted data in duplicate, and conducted fixed-effects meta-analysis.

**Results:** We included seven RCTs which assessed five different vaginal microbicides (namely, Savvy, cellulose sulphate, Carraguard, PRO-2000, and tenofovir) in 23,840 HIV-negative heterosexual women. The pooled results show no evidence of an effect of vaginal microbicides on the risk of HIV acquisition in women (RR 0.94, 95% CI 0.82 to 1.08). However, there was moderate statistical heterogeneity in study results (I²=43%). Tenofovir appears to reduce risk (RR 0.63 95% CI 0.43 to 0.93); but there was no evidence of an effect for cellulose sulphate (RR 1.20, 95% CI 0.74 to 1.95), Savvy (RR 1.38, 95% CI 0.79 to 2.41), Carraguard (RR 0.89, 95% CI 0.71 to 1.11), and PRO-2000 (RR 1.01, 95% CI 0.81 to 1.27). There was no evidence of an effect of vaginal microbicides on acquisition of other STIs. In addition, the microbicides did not increase in the incidence of adverse events.

**Conclusion:** Currently available evidence suggests that vaginal tenofovir (a nucleotide reverse transcriptase inhibitor) microbicides may reduce HIV acquisition in heterosexual women; but other types of vaginal microbicides have not shown evidence of an effect on HIV or STI acquisition. Further studies are needed to confirm the beneficial effects of the tenofovir gel in vaginal and anal sex. In addition, further research should continue on the development and testing of new microbicides.
88. Increased risk of pneumococcal pneumonia among HIV and influenza co-infected patients hospitalised with pneumonia in South Africa, 2009-2010


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Background: The etiological diagnosis of pneumonia is challenging due to inadequate diagnostic tests and therefore the incidence of pneumococcal pneumonia is underestimated. We aimed to identify patients with pneumococcal pneumonia using a polymerase chain reaction (PCR) assay, and to identify risk factors for pneumococcal pneumonia among patients hospitalised with pneumonia.

Method: Patients were enrolled from May 2009 through December 2010 as part of a prospective hospital-based pneumonia surveillance programme covering six hospitals in four provinces in South Africa. Clinical and epidemiologic data were collected. Streptococcus pneumoniae was identified by quantitative real-time PCR detecting lytA from whole blood specimens. Nasopharyngeal swabs/aspirates were tested for influenza virus by real-time reverse-transcription PCR. HIV status was determined by ELISA or PCR depending on patient age. Multivariable logistic regression analysis was performed.

Results: Of the 5411 patients with hospitalized pneumonia enrolled, 379 (7%) tested lytA positive on blood for pneumococci. Pneumococcal prevalence was 5% (78/1714), 6% (25/399), 1% (2/174), 9% (193/2082) and 8% (81/1042) in the <2, 2-5, 6-18, 19-44 and ≥ 45 years age groups respectively. On multivariable analysis lytA positive, compared to lytA negative, patients presented at the hospital later (>2 days from symptom onset) [320/379 (84%) vs 3572/5009 (71%); odds ratio (OR): 1.7, 95% confidence interval (CI) 1.3-2.4], had longer hospitalization time (>5 days) [225/378 (60%) vs 2251/5005 (45%); OR: 1.4, CI 1.1-1.8], had higher rates of HIV infection [257/352 (73%) vs 2443/4594 (53%); OR: 1.9, CI 1.5-2.5] and influenza co-infection [49/377 (13%) vs 451/4997 (9%); OR: 1.6, CI 1.1-2.2] and were at higher risk of dying [38/378 (10%) vs 296/5010 (6%); OR: 1.5, CI 1.1-2.2].

Conclusion: HIV-infection and influenza co-infection are each independent and significant risk factors for pneumococcal pneumonia. Amongst patients hospitalized with pneumonia, individuals with pneumococcal pneumonia present later, have longer hospitalization and have an increased risk of death.