Track 4: Microbiology I (Chemotherapy / Drug Resistances **Venue: Training Center (Room 1)**

3.101

A pilot survey of farmers' motivations of antibiotics use in livestock production in Kenya

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Abstract

Livestock production is a major livelihood source for a large proportion of the population in Kenya. However, its productivity is low partly due to the high incidence of disease. The presence of animal diseases reduces the efficiency of input use in production and also necessitates the use of antimicrobials such as antibiotics. Although there is wide use of antibiotics in livestock in Kenya, the motivations for antibiotic use as well as the use patterns are not well understood. Studies show that inappropriate use of antibiotics is a leading cause of antibiotic resistance which has important implications on human health. A proper understanding of the incentives that drive antibiotic use, therefore, is an important first step in developing effective policies and programs aimed at reducing misuse of veterinary drugs. This study analyzed the motivations for use of antibiotics in livestock production in Kenya. Sixty farmers – 20 farmers in each of three production systems (pigs, poultry and beef cattle) – were interviewed using a pre-tested questionnaire for their knowledge, practices and attitudes of antibiotics use in livestock. The results indicate that antibiotics were often the drug of choice for poultry and beef cattle keepers. Pig farmers reported low disease incidence, hence their low use of antibiotics. Almost all the antibiotics used by farmers in beef cattle were tetracyclines (e.g., Oxytetraclin®, Adamycin®, Oxy-met®), while those used in poultry and pigs were tetracyclines (e.g., Alamycin®), fluoroquinolones (e.g., Furazolodine®) and sulphonamides (e.g., Biotrin®, S-Dime® and Poultricin®). In all the three production systems, the antibiotics were mainly used to treat common ailments; however, in a few cases, antibiotics were also used as growth boosters in poultry. The study provides important information that increases our understanding of the dynamics of antibiotic use and the potential development of antibiotic resistance in livestock in Kenya.

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Antimicrobial resistance and virulence factors in environmental and clinical *Vibrio cholerae*

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Background: There has been an upsurge of cholera cases in Kenya with 3091 cases in 2008 and 11,769 cases (CFR=2.3%) reported to The Ministry of Public Health and Sanitation in 2009.
Objective: To determine the environmental reservoirs of *V.cholerae* during the interepidemic periods and to characterize their antimicrobial resistance and virulence factors.

Methodology: Environmental samples including sediment, algae, brackish water were collected from epidemic prone areas in Kenya which included the coastal region and the lake- basin regions. Isolates from environmental samples were tested for susceptibility to 12 antibiotics. Virulence factors and resistance gene types were determined using multiplex PCR. Archived 2009-

2010 clinical outbreak strains of *V. cholerae* were also characterized along for comparison.

Results: A hundred (50 clinical, 50 environmental)*V. cholerae* isolates were used in the study. Overall, sediment followed by algae from fishing and landing bays led in samples which contained *V. cholerae*. All clinical strains were susceptible to tetracycline while all environmental strains were susceptible to cefuroxime. All clinical strains were resistant to streptomycin, sulfamethoxazole, trimethoprim, 98% resistant to furazolidone, while 92% were resistant to nalidixic acid. Environmental strains were 64%, 60%, 60% resistant to cephalothin, sulphamethoxazole and sulbactam/ampicillin respectively. All clinical strains harboured *ctxA*, *tcpA* (El Tor), *ompU* ,*zot* ,*ace* , *toxR* ,*hylA* (El Tor), *tcpI* genes. Prevalence for virulence genes in environmental strains were *hyl*A El Tor (10%), *toxR*(24%), *zot* (22%), *ctxA*(12%) ,*tcpI*(8%), *hylA*(26%) and *tcpA* (12%). Clinical strains possessed SXT-element which was rare in environmental strains.

Conclusion/recommendations: Study sites including landing bays and beaches contained environmental *V. cholerae*. Presence of resistance to antibiotics and some virulence factors showed pathogenic potential of the environmental isolates and may be reservoirs for frequent epidemics. Improved hygiene and fish handling techniques will be important in prevention of persistence of reservoirs.

3.103

Chloroquine resistance status a decade after; Re-emergence of sensitive *Plasmodium falciparum* strains in malaria endemic and epidemic areas of Western Kenya.

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Introduction: Malaria parasites have become increasingly drug-resistant, and resistance has spread geographically. In 1998, when the clinical efficacy of chloroquine (CQ) against falciparum malaria dropped below 50%, Kenya replaced this agent countrywide with sulfadoxine-pyrimethamine (SP) for first-line treatment of uncomplicated malaria. Earlier in 1993, when the clinical efficacy of CQ against falciparum malaria dropped in Malawi replaced this agent countrywide with SP for first-line treatment of uncomplicated malaria. More than a decade after chloroquine was removed from routine use in Malawi because of parasite resistance to it, the drug is again effective. In Kenya a study that was done in May 2005 in a low malaria transmission area showed that 94% of field isolates from this site still harbored T76 mutation in *Pfcrt* while 6% had the wild type allele K76 giving a P=0.04058 when compared to what was prevailing when the drug was withdrawn from public use in 1997. The present study was aimed at establishing the CQ resistance status in the country, ten years after its withdrawal, by looking at high malaria transmission zone, Mbita, a malaria endemic area and some malaria epidemic areas of the Kenyan highlands.

Findings: The prevalence of T76 and Y86 *Plasmodium falciparum* molecular markers for chloroquine (CQ) resistance in *Pfcrt* and *Pfmdr*-1 genes were investigated by PCR-RFLP and dot blot analysis in 64 samples collected in March to May 2007 in the endemic area and 38 samples collected in April to July the same year in the epindemics, ten years after CQ cessation. The study shows that 67.3% of field isolates from the endemic site still harbor Y86 mutation in *Pfmdr1* while 32.7% have the wild type allele N86 compared to the 94% and 6 % prevalence observed in Mwea, an endemic area, in 2004. χ^2=10.08, P=.00015, 95%CI=2.085-27.8. In the epidemics 75% of field isolates from the epidemic sites still harbor Y86 mutation in *Pfmdr1* while 25% have the wild type allele N86 compared to the 91.6% and 8.4% prevalence observed in an epindemic area in 1997. χ^2=1.585, P=.208, 95%CI=.701-19.176.

Conclusion / Recommendation: From the study there is a significant change in the proportions of the resistant genotypes in the endemic areas while

in the epidemics there was also a noticeable shift though not significant. This therefore indicates a slow but steady re-emergence of *P. falciparum* chloroquine sensitive strains in the country.

3.104

Pharmacokinetics/Pharmacodynamic of Amodiaquine and Desethylamodiaquine in Patients with malaria

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Background: Amodiaquine (AQ) is an antimalarial drug that is frequently combined with artesunate (AS) for the treatment of uncomplicated malaria due to *Plasmodium falciparum* and is now available as a fixed dose combination. Despite its widespread use, the simultaneous pharmacokinetics of AQ and its active metabolite, desethylamodiaquine (DAQ) were not characterized to date in patients.

Methods: The pharmacokinetics of AQ and DAQ were therefore investigated in 54 adult patients in Chulaimbo Sub-District Hospital receiving the co-packaged and FDC AS/AQ for the treatment of uncomplicated malaria. Population pharmacokinetic approach was used.

Results: AQ followed a 1-compartment model with first-order absorption and elimination as well as a first-order and irreversible transformation into DAQ, which in turn followed a 2-compartment model with first-order elimination from its central compartment. Mean AQ apparent clearance and distribution volume were 3410 L/h and 39200 L respectively. Mean terminal elimination half-life of DAQ was 211 h. Bodyweight was found to explain the interindividual variability of the apparent distribution volume of AQ and the elimination rate constant of DAQ. A new dosage form consisting in a fixed dose combination of AS and AQ was found to have no effect on the pharmacokinetic parameters of AQ and DAQ, and the AUC's of both for the new FDC were comparable to the ones of the two drugs co-administered as separate tablets.. All patients achieved parasite eradication within 4 days following the onset of the treatment, which prevented the investigation of the possible relationship between DAQ exposure and treatment outcome.

Conclusion: This study provided the first simultaneous pharmacokinetic model for AQ and DAQ.

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Selective Reversal of Piperaquine and Lumefantrine Resistance in *Plasmodium berghei* ANKA

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Background: Drug resistance against *Plasmodium falciparum* remains a public health problem. Strategies to overcome this problem require full understanding of the resistance mechanisms. We used *Plasmodium berghei* as a surrogate to *Plasmodium falciparum* to study antimalarial resistance. Stable lumefantrine (LM) and piperaquine (PQ) resistant *P. berghei* selected by *in vivo* drug pressure were used. To further understand the resistance mechanisms, we have tested the ability of known *P. falciparum* reversing agents, probenecid, verapamil and cyproheptadine to reverse LM and PQ resistance.

Methodology: Chemo-sensitization potential of probenecid, verapamil or cyproheptadine was assessed in 4-day test in which *P. berghei* in mice is exposed to four, daily drug doses. Oral treatment with LM, PQ alone or in combination with chemosensitizer was administered for a total of four daily doses. Parasite density was estimated microscopically (×100) 96 hours post parasite inoculation using thin blood films.

Results: Parent strain was sensitive to LM and PQ with an ED_{90} of 3.52 and 3.93mg/kg respectively. Lumefantrine resistant (LM^R) and piperaquine resistant (PQ^R) obtained after 1-2 years of drug pressure had ED_{90} of 52.06 and 63.39mg/kg respectively. We first tested the reversing agent alone to identify the highest doses that do not inhibit parasite growth and these doses were used to carry reversal experiments. At 5mg/kg, cyproheptadine restored LM activity by 65% against LM^R but failed to restore PQ activity against PQ^R. Probenecid (400mg/kg) and verapamil (50mg/kg) did not chemo-sensitize either LM^R to LM, or PQR to PQ. A previous study showed that PQ^R is also resistant to LM (ED_{90} 97.25mg/kg). Interestingly, these 3 chemosensitizers restored LM potency against PQ^R.

Conclusion: Our data shows the potential of cyproheptadine to restore LM activity in LM^R and also indicate that the selection of PQ^R is associated with LM decrease efficacy, however this efficacy can restored by chemosensitizers.

Key words: *Chemosensitizer; Resistance; Piperaquine, Lumefantrine, Plasmodium berghei*

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The *Pfmdr1, Pfcrt conspiracy; a Mefloquine, Lumefantrine and Chloroquine Triangle.*

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Background: Polymorphisms in the Pfmdr1 and Pfcrt genes of Plasmodium falciparum are associated with resistance to several anti-malaria drugs. PfMDR1 86Y and PfCRT 76T, are associated with chloroquine resistance, suggesting multiple molecular mutations confer chloroquine resistance. In contrast, PfMDR1 86Y is associated with increased mefloquine and lumenfantrine sensitivity. Lumefantrine likely selects for parasites with PfCRT K76.

Objective: To investigate the role of Pfmdr1 86 and Pfcrt 76 in P.falciparum resistance to mefloquine, lumefantrine, and chloroquine.

Methods: Using molecular genotyping and the malaria SYBR Green I Fluorescence-based assay, we examined Pf field isolates collected in malaria endemic sites in western Kenya and Malindi for mutations in the PfMDR1 and PfCRT genes and *in vitro* drug sensitivity profiles (IC50) for selected anti-malarials, respectively.

Results: Isolates with higher mefloquine IC50s lacked the pfmdr1 86Y mutation, whereas those with the mutation had lower IC50s ($p = 0.0309$). Isolates with higher lumefantrine IC50s lacked the PfCRT 76T mutation, whereas those with lower IC50s contained the mutation ($p = 0.0163$).

Conclusion: These results suggest that mefloquine significantly selects for parasites with PfMDR1 N86 wild type. We also observe a trend of lumefantrine selection of the PfMDR1 N86 wild type. Therefore, as chloroquine pressure on the parasites decreases and more parasites revert to PfMDR1 N86, we expect a continued increase in mefloquine and lumefantrine resistance. Additionally, the data indicates that lumefantrine selects for PfCRT K76 wild type. Since lumefantrine is a partner in Coartem, the first-line antimalarial in Kenya for uncomplicated Malaria and its future use may increase, we suggest that the PfCRT 76T mutation will be maintained in most parasites as they respond to continued lumefantrine pressure. However, should the parasites select for PfCRT K76, as a result of the decreasing chloroquine pressure, lumefantrine susceptibility may be lost and result in decreased Coartem efficacy.

3.107

Molecular characterization and antimicrobial resistance of enterococcal isolates from Aga Khan Hospital, NairobI.

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Background: *Enterococcus* spp. account for a large number of nosocomial infections. Resistance for *Enterococcus* spp. is on the increase, this greatly compromises the choices of antibiotics available to treat infections caused by these bacteria.
Objective: To determine the prevalence, antimicrobial resistance patterns and resistance genes in *Enterococcus faecium* and *Enterococcus faecalis* isolates from patients at the Aga Khan University Hospital (AKUH) Nairobi.
Methodology: All consecutive clinically significant enterococcal isolates, collected at AKUH in the months of March 2007 to February 2008 were used**.** Species identification was done using API 20 STREP. Susceptibility testing was done using Disk diffusion and Minimum Inhibitory Concentration (MIC). Interpretation of the Susceptibility results was done using the Clinical and Laboratory Standards Institute (CLSI) guidelines. Resistance gene detection was done for tetracycline (*tet M* (696bp)), Floroquinolones (*gyr A* (241bp), and Chloramphenicol (**cat_{pIP501}** gene (540bp)) using PCR.
Results: *Enterococcus faecalis* was the most prevalent 128(85%) species, followed by *Enterococcus faecium* (5%). Both species were fully resistant to aminoglycosides and tetracyclines while glycopeptides were the most effective. 75.9% and 100% of the *Enterococcus faecalis and Enterococcus faecium respectively* were positive for the *gyrA* gene. 61.8% and 60% of the *Enterococcus faecalis and Enterococcus faecium respectively* were positive for the *tet M* gene. 63% of the *Enterococcus faecalis* and 100% of *Enterococcus faecium respectively* were positive for the **cat_{pIP501}** gene.
Conclusion: With the high levels of resistance to aminoglycosides and tetracyclines and emerging resistance to fluoroquinolones*, routine susceptibility testing is required before treatment is instituted using commonly available drugs in the hospital.

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Comparison of PCR and phenotypic detection methods for Methicillin resistant *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* is one of the most significant human pathogens causing both nosocomial and community acquired infections. *Staphylococcus aureus* can cause a range of infections from mild to life threatening conditions such as pneumonia. Strains of MRSA have spread between different hospitals, cities, countries, and even continents and are now the cause of hospital infections. Resistance to methicillin and other beta lactam antibiotics is mediated by the over production of an additional altered novel penicillin binding protein (PBP) (PBP2a or PBP2`)
Objectives: The broad objective was to compare between phenotypic detection methods for MRSA and polymerase chain reaction (PCR) assay in clinical isolates.
Methods: A total of 100 *Staphylococcal* clinical isolates were initially identified and confirmed by API and then subjected to E-Test MIC (oxacillin and cefotaxime), MRSA Latex agglutination test and PCR assay for *Mec A gene* as the "gold standard" for *MRSA* detection.
Results: Out of the 100 isolates, 31% were (MRSA) resistant to both oxacillin and cefotaxime, while MRSA detection by latex agglutination and PCR was 37% and40% respectively. All the isolates from lung aspirates showed very high resistance of >256 for both oxacillin and cefotaxime while wound swabs recorded the highest percentage of MRSA with HVS and CSF recording none.
Recommendations /Conclusions: There is need to put in place strategies to strengthen and sustain public health laboratories to be able to diagnose and identify MRSA correctly. Infection-control program should be prioritized in all public health facilities. Early and specific diagnosis of MRSA infections is important in preventing their spread.

MRSA prevalence is best predicted by use of PCR in epidemiological studies but phenotypic methods are suitable when it comes to immediate treatment and management of *Staphylococcal* infections.

Track 5: Microbiology II **Venue: Training Center (Room 1)**

3.201

Review of drug susceptibility testing (DST) practices for TB re-treatment cases in Kenya

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Background: Multi-drug resistant tuberculosis (MDR-TB) threatens to reverse gains Kenya has made in achieving WHO TB control targets. However, the actual burden of MDR-TB remains unknown. TB re-treatment cases represent a cohort with the highest risk for MDR-TB. Standard clinical care for re-treatment cases includes drug susceptibility testing (DST) which, if optimized, could provide valuable MDR-TB surveillance data. In this study, we reviewed DST request practices for TB re-treatment cases in Kenya.
Methods: We reviewed national registration records of TB re-treatment cases in 2006 and compared these to DST data at the national TB central reference laboratory (CRL). A more in-depth audit was conducted in Nyanza Province and at the CRL to identify logistic obstacles that need to be addressed to optimize DST coverage.
Results: DST were performed for 2511 (24.4%) of the 10,462 TB re-treatment cases registered in 2006. Over the same period, Nyanza Province registered the lowest DST coverage rate of 10% (200 DSTs for 2000 re-treatment cases) compared to the other seven Provinces. Nairobi Province registered the highest coverage rate of 53% (1238 DSTs for 2341 re-treatment cases). Several logistic obstacles accounting for the low coverage in Nyanza were identified. These included lack of basic supplies and standard operating procedures (SOPs), suboptimal field supervision, and communication gaps between regional TB clinics and the CRL
Conclusions and Recommendations: DST coverage for TB re-treatment cases is suboptimal and may lead to underestimation of the MDR-TB burden. As part of the efforts to improve the national MDR-TB surveillance system, Kenya must address logistic obstacles and expand DST coverage for TB re-treatment cases.

3.202

Tuberculosis Treatment Interruption in Nairobi, Kenya; A treatment supporter perspective.

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Background: Nairobi, the capital city of Kenya has an estimated population of 3,100,000 people in an area of 696 sq. km. with about 60% of the people living in only 5% of the total area. Tuberculosis (TB) diagnosis and treatment is provided in accordance with the DOTS strategy through 207 TB treatment sites (119 public and 88 private). Of the 18,500 TB patients started on treatment from 1st January to 31st December 2008, 1,429 (7%) defaulted (i.e. interrupted treatment for two consecutive months or more). Treatment interruption and subsequent default from treatment

results in persistent transmission of tubercle bacilli within the community, increased morbidity and cost to TB control programmes. It can also lead to relapse and drug resistant TB, and is one factor responsible for the persistence and resurgence of tuberculosis especially multi-drug resistant TB (MDR TB).

Objective: To document reasons for interruption of treatment by TB patients.

Methods: This was a cross-sectional descriptive study. Tuberculosis patients registered for treatment in 119 public TB treatment sites between 1st October 2008 and 31st March 2009 were eligible and each patient was assigned a treatment supporter who supervised the treatment. The patients collected medicines at weekly appointment clinics during the intensive phase and two-weekly during the continuation phase. If a patient missed two consecutive drug collection clinics, the health worker traced the patient or the respective treatment supporter to find out the reason for the patients failure to collect medicines. Structured questionnaires were used to collect data which were administered by the health care workers. The questionnaires were later serialized and captured into the electronic database using MS Access. Double entry method of data for data entry was applied and SPSS™ used for analysis.

Results: Sixty-eight percent (68%) of the patients interrupted treatment during the intensive phase (i.e. within two months after commencing treatment). Of the 397 patients whose treatment supporters were traced, reasons for interruption included; travelling out of Nairobi (30%), referral around and out of Nairobi (15.4%), dead (8.3%), drug side effects (6.5%), assumed cure (6.5%), hospitalization (6.3%), too ill (5.8%), work related (4%), imprisonment (3%), drug abuse (2.2%), and other reasons (12%).

Conclusion: In Nairobi, TB patients mostly interrupt medication during the intensive phase and frequent movement (travelling out of Nairobi) is the main cause of treatment interruption.

Recommendation: Appropriate patient education, including information on treatment duration and possible outcomes should be provided repeatedly during drug collection clinic days. Where a patient misses the drug collection clinic day the health worker should immediately trace either the patient or the treatment supporter to find out the true status of the patient and outcome recorded or appropriate action taken to ensure treatment continued. In addition, the existing referral systems need to be strengthened in order to confirm the treatment outcomes of all referred patients.

Acknowledgements: District public health officers, Nairobi Province.

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High ethionamide resistance in *mycobacterium tuberculosis* strains isolated in Kenya.

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Background: Increasing development of tuberculosis (TB) resistance to the currently available drugs including second-line anti-TB drugs that are being used for treatment of Multi-Drug Resistant TB (MDR-TB) patients has frustrated efforts to control TB worldwide. Ethionamide (Eth) is one of the drugs used in the regimen for treatment of these patients.

Objective: To determine levels of resistance among second-line anti-tuberculosis drugs in Mycobacterium tuberculosis (MTB) strains isolated in Kenya.

Design: A retrospective lab-based study involving archived strains from previous studies carried out at the Centre for Respiratory Diseases Research (CRDR), Kenya Medical Research Institute (KEMRI) from 2002 to 2007.

Setting: CRDR, KEMRI.

Methods: A total of 216 MTB strains with pre-determined first-line drug susceptibility testing (DST) results were used including 78 first-line resistant to individual and combined drugs, and 138 susceptible to streptomycin, rifampicin, isoniazid and ethambutol. The strains were subjected to DST to ethionamide among other second-line.

Results: Thirty two [32/216 (14.8%)] strains showed resistance to second-line drugs. Resistance to Eth was the highest [18/32 (56.3%)] including co-resistance with isoniazid [8/18 (44.4%)]. Nine [9/18 (50%)] strains were fully resistant and 9 [9/18 (50%)] were intermediate resistant to Eth.

Conclusion: Unexplainable high levels of Eth resistance is a cause for concern. This will impact

negatively on the outcome of management of MDR-TB especially in Kenya where the use of this drug is almost mandatory. Close monitoring of Eth before initiating individual patient management may be necessary.

Key words: Ethionamide, Resistant, MDR-TB.

3.204

Diversity of *Mycobacterium tuberculosis* strains in Nairobi, Kenya.

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Background: Nairobi, the capital city of Kenya has a steadly growing population due to rural-urban migration and immigration. The population in the year 2010 was about 3.1 million people according to the 2009 national population census. The total area of Nairobi is 696 sq km and 60% of the people live in less than 5% of this area in overcrowded informal settlements in form of shelters. In 2007 Nairobi reported 19,842 all forms of TB (684/100,000 population) and 6,634 new smear postive. Some *M. tuberculosis* strains such as the Beijing family have been shown to cause epidemics of multi-drug resistant tuberculosis. It is therefore important to study the genotypic characteristics of *M. tuberculosis* strains that fuel the TB epidemic in Kenya especially Nairobi.

Objective: To determine *Mycobacterium tuberculosis* strains families circulating in Nairobi.

Methods: *Mycobacterium tuberculosis* isolates were obtained from sputum specimens collected from consecutive new and previously treated pulmonary smear positive patients between February and August 2010 and cultured on Lowenstein-Jensen media. Spoligotyping was done on DNA extracted from the first isolate of each patient. The international spoligotype data base (SpolDB4) was used to assign isolates to strain families.

Results: Fourty seven different strain families were identified from 536 isolates. These could be grouped into; CAS1_KILI 96/536 (17%), T1 9/536 (12%), Beijing 65/536 12%), LAM9 46/536 (9%), LAM3 & S/Conversant 37/536 (7%), LAM11_ZWE 26/536 (5%), CAS1_DELHI 24/536 (4%) , T2 24/536 (4%) and others previously identified 113/536 (21%). A possible new family subtype was identified with 21/536 (4%) isolates. Others types not previously identified accounted for 15/536 (3%).

Conclusion: We found a diverse array of strains which could be indicative of a cosmopolitant polulation with frequent migration. This may suggest that the dorminant genotypes may have been present in the population for an extended period of time or on going transmision of closely related strains. We therefore call for strenghthening efforts on early case finding through enhanced public health education campains and provision of accessible diagnostic services with enhanced treatment compliance.

3.205

A Prospective Cohort Study To Evaluate Incidence Of Tuberculosis In Infants, Western Kenya

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Background: There is paucity of age specific TB incidence data among infants in Kenya. New TB vaccines are likely to be administered to infants. Therefore, cohort studies which include comprehensive diagnostic methods to provide reliable estimates of TB incidence and other epidemiological parameters in infants are needed to guide the planning of future TB vaccine trials. We set out to determine the incidence of TB, latent TB infection and all cause and TB specific mortality rates in Siaya district, western Kenya.
Objective: Determine the incidence of definite and probable TB in infants
Methods: To demonstrate a TB incidence of 0.5%, and make inferences for a phase III trial, ~2900 infants are being enrolled and followed up for a minimum of one year. Through 4-monthly follow up visits and health facility (HF) surveillance, those determined to be TB suspects (n=199) by history of contact, TB symptoms, hospitalisation history, are admitted to a case verification ward. Specimens are collected for microscopy and culture by induced sputum and gastric aspiration. Chest radiographs, mantoux tests, and HIV testing are performed. Morbidity and mortality surveillance is conducted through HF record reviews.
Results: 2900 BCG vaccinated infants were enrolled with cumulative follow up of 1352 person years by November 2010. Of 2900 participants, 454(15.6%) were TB suspects of which 353/453(77.8%) had been investigated for tuberculos. 23 TB cases were diagnosed giving a TB incidence of 1.7(95% CI 1.08-2.54) cases per 100 person years.
Conclusion: Tuberculosis (TB) in young children is under-diagnosed in resource-constrained settings. The results demonstrate that TB is a major public health problem in this age group and indicates the suitability of our site for TB vaccine trials in infants.

3.206
The prevalence of tuberculosis infection and disease among adolescents in western Kenya: preparation for future TB vaccine trials.
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KEMRI/CDC Research and Public Health Collaboration

Introduction: The risk of tuberculosis (TB) disease following infection begins to increase in adolescence, which makes this high risk population a target for new TB vaccines. In order to contribute to the design and implementation of future TB vaccine trials, we sought to determine the incidence and prevalence of TB infection and disease among adolescents in Western Kenya.
Methods: A prospective cohort study of 5000 adolescents aged 12-18 years is being conducted by KEMRI/CDC in Western Kenya in an area under continuous demographic surveillance. Adolescents are enrolled and followed for one year. TB suspects are defined using clinical criteria, history of contact with a TB case and/or a positive mantoux [(TST); TST positive = >=10mm or >=5mm if HIV+]. Suspects are investigated for pulmonary tuberculosis (PTB) through sputum examination (microscopy and culture), and chest radiography.
Results: Out of 5004 adolescents enrolled by August 2009, 2425 (48.5%) were female and 2579(51.5%) males, median age is 14 years. 2504 (50%) were identified as TB suspects with 1521 (85.6%) having one trigger for being TB suspects i.e. either clinical symptoms, positive TST or history of contact; 206 (11.6%) and 18(1.01%) had 2 and 3 triggers respectively. 1170 (65.8%) had a TST > 10mm. 15 cases with definite PTB were identified (positive smears or culture) and 7 with probable PTB based on clinical and radiological criteria, reflecting crude prevalence estimates of 300/100,000 (definite) and 440/100,000 (definite and probable) PTB respectively.
Conclusion: The prevalence of TB infection and pulmonary TB disease among adolescents in western Kenya appears quite high. These preliminary results suggest that this study population will be a suitable target population for TB vaccine trials.

3.207
Retention Patterns of an Adolescent Cohort in Western Kenya in Preparation for Future Vaccine Trials
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Background: The risk of tuberculosis (TB) disease following infection begins to increase in the age range 12-18 years. This high risk population group is therefore a prime target for new TB vaccines. Ability to track and retain an adolescent cohort are essential prerequisites to conducting future vaccine trials in this age group
Objectives: To estimate the prevalence and incidence of TB among adolescents in Western Kenya
Methods: 5004 adolescents aged between 12-18 years were enrolled for one year and followed up every four months for one to two years. Efforts were made to locate participants moving out of the study area. Descriptive statistics were used to calculate the monthly retention percentages while bivariate (Pooled t-test for equal variances) analysis was used to test for differences in mean retention rates at the 95% confidence level using 12-14 year olds as the baseline group and the other age groups as the comparison groups. This analysis was also done comparing males and females.
Results: The average retention for this cohort was 79%. Retention was similar between male and female adolescents (p=0.24).There was a significant difference in retention between 12-14 (85.21%), 15-17(72.15%) and 18 (59.71%) year olds (P=0.0022).
Discussion and conclusion: Younger adolescents showed a better retention compared to older adolescents. Our data reveal that retaining an adolescent cohort for a TB vaccine trial is possible in Western Kenya when proper tracking mechanisms are put in place as adolescents are highly mobile.

3.401

PNEUMOCOCCAL PROTEIN VACCINES: USING DNA MICROARRAYS TO IDENTIFY NOVEL PROTEIN ANTIGENS

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Streptococcus pneumoniae (the pneumococcus) continues to be a leading cause of morbidity and mortality worldwide in both children and adults, despite the availability of pneumococcal vaccines. This can be explained partly by the fact that the available vaccines either do not elicit long-lasting protection or are limited in strain coverage. A protein antigen immunogenic in infants and with a conserved epitope across all serotypes would overcome some of the limitations of the current vaccines. The aim of this work was to find novel proteins that might be used as future vaccine antigens. A vaccine that prevents adherence/colonization would naturally prevent the development of subsequent more serious infections.

We used a combination of adherence assays, transcriptomics (oligonucleotide-based DNA microarrays) and functional genomics (insertion-deletion mutagenesis) to identify genes involved in adherence. Two pneumococcal strains were used in the study.

The adherence assays showed that both pneumococcal strains used in the study adhered and invaded the nasopharyngeal cells, though with varying capacities. Upon interaction with nasopharyngeal cells, both strains altered their gene expression profiles relative to control bacteria. In total, 213 genes were up-regulated by adherent pneumococci and 183 were down-regulated. A high proportion of genes of unknown function were up-regulated suggesting the need for further characterization. The function of a few over-expressed genes was further analyzed using insertion-deletion mutagenesis. The ability of mutants to adhere to human nasopharyngeal cells

was compared against the wild-type strains. Five mutants representing novel genes were, to different degrees, attenuated in adherence.
This study provides insights into the shared and distinct mechanisms involved in mediating adherence of two pneumococcal strains to epithelial cells. We have identified genes that play an important role in adhesion, and therefore potential targets for vaccines against pneumococcal colonization.

3.402

Safety of the Oral Pentavalent Rotavirus Vaccine in Kenya, including among HIV Infected Infants

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Background: To evaluate the pentavalent rotavirus vaccine (PRV), RotaTeq®, in Africa, a multicenter Phase III trial was conducted in Ghana, Mali and Kenya from April 2007-March 2009. The vaccine had not previously been evaluated in Africa; we assessed safety of the vaccine in Kenya study participants, including among HIV-infected infants.
Methods: In rural western Kenya, 1,308 infants were randomized (1:1) to receive 3 doses of PRV/placebo at approximately 6-, 10-, and 14-weeks of age. HIV counseling and testing was offered; HIV infection was not an exclusion criterion. All severe adverse events (SAE) at Day 7 and 14 following each dose, and vaccine-related SAEs and all deaths occurring at any time during the study, were reported. The first 297 consenting participants were also followed for 42 days after any dose for any adverse event (AE), regardless of severity (intensive safety surveillance).
Results: SAEs were reported in 20/649 vaccine recipients (3.1%) and 21/643 placebo recipients (3.3%) within 14 days following vaccination (p=0.9). The most common SAE in the vaccinated group was pneumonia (1.7%). No specific SAE (including intussusception) was more common among PRV than placebo recipients. Sixty-six deaths were reported in Kenya, 36 (5.5%) and 30 (4.7%) among PRV and placebo recipients, respectively (p=0.47). In intensive safety surveillance, 137/147 (93.2%) vaccine recipients and 147/150 (98.0%) placebo recipients experienced one or more AEs. 89.2% of the infants were tested for HIV infection; 19/587 (3.2%) children in the PRV group and 11/580 (1.9%) in the placebo group were HIV-infected. Among the 30 HIV-infected infants, 5/19 (26%) PRV recipients and 1/11(9.1%) placebo recipients reported a non-death outcome SAE (p=0.4); 8 of 19 (42%) HIV-infected infants receiving PRV died versus 3 of 11 (27%) receiving placebo (p=0.5).
Conclusions: PRV appears to be a safe intervention against rotavirus gastroenteritis among infants in Kenya. AEs, including severe ones, did not occur more frequently in PRV recipients compared to placebo recipients. The number of HIV-infected participants in the trial provided insufficient power to assess whether SAEs occurred more frequently in HIV- infected vaccine recipients.

3.403

Etiology of diarrhea and its sequelae among children <5 years old in rural western Kenya, 2008-2010

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Background: Diarrhea is a leading cause of childhood morbidity and mortality in Kenya. **Objective**: To characterize etiology for diarrhea and its sequelae among children <5 years in western Kenya.
Methods: We conducted a case-control study of moderate-to-severe diarrhea among children <5 years old in western Kenya as part of the Global Enteric Multicenter Study (GEMS). A case, enrolled at a clinic within 7 days of illness onset, was defined as ≥3 loose stools in 24 hours with ≥1 characteristic: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization. We enrolled age-, gender- and village-matched controls at home. We re-assessed health outcomes after ~60 (range 50-90) days.
Results: From January 28, 2008, to August 26, 2010, 1,338 cases and 1,674 matched controls were enrolled. Pathogens identified among cases and controls included: rotavirus (13.5% vs. 1.7%, matched odds ratio (mOR) = 10.8; 95% confidence interval: 6.5–17.8), *Shigella* (7.7% vs. 3.7%, mOR = 2.5 [1.7–3.5]), *Cryptosporidium* (10.8% vs. 5.0%, mOR = 2.4 [1.8–3.3]), nontyphoidal *Salmonella* (5.7% vs. 3.3%, mOR = 1.8 [1.2–2.5]), enterotoxigenic *Escherichia coli* (15.5% vs. 9.9%, mOR = 1.7 [1.3–2.1]), enteropathogenic *E. coli* (11.2% vs. 8.6%, mOR = 1.3 [1.0–1.6)], and *Campylobacter* (13.6% vs. 12.7%, mOR = 1.06 [0.9–1.3]). By ~60 days, 4.1% cases vs. 0.7% controls had died (p<0.01). Cases who died were more likely than those who survived to have had clinical features of malnutrition (flaky skin (28% vs. 2%, OR = 17.7 [8.5–36.7), abnormal hair (31% vs. 4%, OR = 10.2 [5.3–19.7]), bipedal edema (16% vs. 1%, OR = 18.3 [7.1–47.1]), and wasting (49% vs. 8% OR = 10.7 [6.0-9.3]) at enrollment.
Conclusions: The leading pathogens associated with moderate-to-severe diarrhea were rotavirus, *Cryptosporidium* and *Shigella*. A variety of indicators of malnutrition were predictive of fatal outcomes.

3.404

Sequence type 313 S. Typhimurium dominates in invasive non-typhoidal Salmonella infections in children, Kenya

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Introduction: Whereas most nontyphoidal Salmonella (NTS) are associated with gastroenteritis, Salmonella enterica serovar Typhimurium isolates are responsible for a significant proportion of invasive bacterial infections in children less than 5 years of age in Kenya, being second in importance only to invasive pneumococcocal disease. NTS specific-cause mortality is estimated at 28%; the problem is compounded by emergence of multidrug resistant strains.
Methods: Over the last 15 years, we conducted sentinel surveillance on causes of bacteremia in children under 5 years of age admitted to hospitals in Nairobi and Kilifi District Hospital. We obtained data on socioeconomic status, clinical presentation and serotype distribution of NTS. Antibiotic susceptibility of NTS isolates was performed by the MIC method and genetic relatedness of isolates was determined by pulsed field gel electrophoresis (PFGE), sequence typing (ST) and full genome sequencing.
Results: Of all cases of admissions with febrile illness (>5000 blood cultures) NTS constituted 18% and resulted in 28% mortality compared to 5.7% mortality in children that did not have bacteraemia (p<0.001), with *S.* Typhimurium as predominant serotype (59%) with 92% being of ST 313. Some 45% NTS were resistant to 3 or more antibiotics, and out of these 59% were resistant to ampicillin, chloramphenicol and tetracycline; rates were significantly lower for isolates from rural population (p<0.001). Whole-genome sequencing identified a distinct prophage repertoire and a composite genetic element encoding MDR genes located on a virulence-associated plasmid in the ST313 strains, with evidence for microevolution and clonal replacement in the field.

Conclusion: The characterization in Kenya of unique ST313 MDR *S.* Typhimurium associated with an epidemic increase in incidence of invasive life-threatening NTS infections will form a basis for gene-based vaccine development. There is also need for continuous surveillance and monitoring of this new genotype as an important cause of community-acquired infections.

Track 4 & 5: Microbiology I & II **Venue: Training Center (Foyer)**

3.109

Serotype and Drug Susceptibility of *Vibrio cholerae* Isolated in Kenya and Southern Sudan, 2005-2009

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Background: In the last decade, sub-Saharan Africa has come to account for the majority of reported cholera epidemics in the world. In recent years, large epidemics of cholera in this region have caused substantial morbidity and mortality. Since 2005, cholera outbreaks, some with high case-fatality ratios, occurred in multiple locations in Kenya; cases identified during 2009 increased by nearly 10-fold over previous years.

Objectives: Serotypes and drug susceptibility data are needed to contribute to effective intervention strategies.

Methods: From June 2005 to December 2009, two hundred and twenty one randomly collected stool specimens among initial cases of suspected cholera from outbreaks from various regions in Kenya and Juba, Sudan, sent to KEMRI-CDC laboratories were cultured; *V. cholerae* isolates underwent drug susceptibility testing by the disk diffusion technique according to CLSI guidelines. Resistance rates were compared over the time period.

Results: *V. cholerae* O1 Inaba was isolated from 104 (47%) stool specimens. Most strains were susceptible to ciprofloxacin (100%), tetracycline (99%), ceftriaxone (99%) and ampicillin (81%). However, high proportions were resistant to nalidixic acid (84%), furazolidone (97%), streptomycin (97%), sulfisoxazole (100%), and co-trimoxazole (100%). Intermediate susceptibility to ampicillin increased over the years from 0% to 37%. All (4) ampicillin-resistant strains were isolated from 2007 to 2009. There were no cholera outbreaks samples in the year 2006 to include in the analysis.

Conclusion: While routine antimicrobial therapy for cholera is not recommended, tetracyclines and quinolones currently may be useful when indicated for treatment of severe cases; however, resistance to nalidixic acid (a step towards clinical resistance to fluoroquinolones) and recent appearance of ampicillin-resistant strains suggest that effective treatment of cholera may become more challenging. Continued surveillance for drug resistant *V. cholerae* isolates is important in Africa in the setting of continued, and perhaps increasing, disease burden.

3.309

Demonstrating resistance-mitigating effect of *artemisia annua* phytochemical blend with in-vitro cultures of *plasmodium falciparum* and in-vivo with *plasmodium berghei* anka in Mice

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Resistance of *Plasmodium falciparum* to drugs such as chloroquine and sulfadoxine-pyrimethamine is a major problem in malaria control. Artemisinin derivatives, particularly in combination with other drugs, are thus increasingly used to treat malaria, reducing the probability that parasites resistant to the components will emerge. Although stable resistance to artemisinin has yet to be reported from laboratory or field studies, its emergence would be disastrous because of the lack of alternative treatments.

The project was designed to demonstrate resistance-mitigating effects of phytochemical blend of *Artemisia annua* relative to pure artemisinin against the malaria parasite *Plasmodium falciparum* and on rodent malaria parasite *Plasmodium berghei Anka.* For the *in vitro* experiments selection was undertaken on two cultures of *P. falciparum* D6 (CQ-sensitive strain from Sierra Leone) and W2 (CQ-resistant strain from Indochina), by exposing them to *A. annua* phytochemical blend and the pure artemisinin over 50 cycles at doses initially required to give 50% mortality (IC_{50}) of the parasites. Dose-response effects of the blend and the pure compound were determined after 10, 20, 30, and 40, cycles and compared to see if significant difference developed in their efficacy in causing mortality of the parasites.

The in vivo experiments mice have been done by inoculating the Swiss mice with the *P. berghei* ANKA parasite and thereafter treated them with the test drugs. After 4 days the mice were passaged and parasitaemia determined to calculate the ED_{50} and the ED_{9O}. The ED_{50} and ED_{90} got for artemisinin with *P. berghei* ANKA was 1.43 and 7.18 mg/kg.day respectively while the ED_{50} and ED_{90} got for the blend with *P. berghei* ANKA was 34.5 and 118 mg/kg.day respectively. The chloroquine resistant murine plasmodium (p yoelii) was also tested using artemisinin and ED_{50} and ED_{90} got was 11.63 and 29.8 mg/kg.day respectively. The efficacy of dihydroartemisinin was also determined in order to compare with artemisinin and the ED_{50} and ED_{90} got for artemisinin with *P. berghei* ANKA was 1.73 and 8.31 mg/kg.day respectively.

3.310

In-vitro Antimicrobial Properties of Three Medicinal Plants from Kilifi District - Kenya

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Background: Microbial infections are on the rise due to HIV pandemic as they form the bulk of opportunistic infections. Bacterial and fungal infections are the main challenges in these individuals.

Objectives: The aim of this study was to evaluate antimicrobial potential and toxicity of the methanol extracts of *Hosludia opposita, Rhus natalensis* and *Combretum illairii*.

Methodology: The plants were collected from Kilifi District and authenticated at the East African Herbarium. Samples collected were dried, ground into fine powder and extracted in methanol. Quantitative bioassay was done using disc diffusion method; minimum inhibition concentration was done using broth dilution methods. The isolates used were *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans*

and *Trichophyton mentarophyte*. Phytochemical screening was done using thin layer chromatograpy and cell toxicity was done using human embryonic lung cells.
Results: *H. opposita* and *C. illairii* were positive for terpenoids, flavonoids and anthaquinones. All the extracts were safe to the mammalian cells with no apparent cytotoxic effect at 100 mg/ml and also at 500mg/ml. *Combretum illairii* plant extracts had good activity against *S. aureus* and *P. aeruginosa* with inhibition zones diameters of 15.60 mm and 17.00 mm respectively. *Rhus natalensis* extracts had moderate activities with inhibition zone diameters of 11.6mm. *Combretum illairii* leaves extracts had the least minimum inhibition concentration of 3.125mg/ml against *S. aureus* and the highest MIC against *P. aeruginosa* of 12.50 mg/ml. *Rhus natalensis* had an MIC of 6.25mg/ against both *S. aureus* and *P. aeruginosa*.
Conclusion/recommendation: The plant extracts in this study has high activity against bacteria strains as compared to fungal. The results validate the ethnobotanical use of the studied medicinal plants. Analysis and isolation of the compounds present as well as determination of their bioactivity of the pure compounds should be done and also conservation campaign should be carried out.

3.406
Pathogenic bacteria in chronic paediatric otitis media
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Introduction: Chronic suppurative otitis media (CSOM) is a non healing condition of a perforated tympanic membrane associated with chronic inflammatory changes of the muco-periostenum of the middle ear resulting in mucoid or mucopurulent otorrhea of more that three months. Children with CSOM are highly susceptible to intracranial complications and infections such as meningitis, mastoiditis and brain abscesses.
Objective: The study was aimed at determining the type and antibiotic susceptibility profile of bacterial pathogens causing CSOM in HIV+ and HIV- children in Nairobi, Kenya.
Design: cohort institutional based study.
Methodology: Sterile swabs were used to collect the exudates from the middle ear. The specimens were transported in CaryBlair media. The swabs were inoculated on chocolate, blood and bromothymol blue agar and incubated appropriately. Isolates were identified by standard methods. Susceptibility to nine commonly used antimicrobial agents was performed using the standard disk diffusion method according to the CLSI guidelines.
Results: A total of 31 children were recruited. Twenty-two were HIV+ and 9 HIV- with age range of 4 months to 16 years (Mean 5.7). Fourteen (45.16%) were 0-5 years and 21 (67.75%) were within 10 years of age. Of all specimens studied 28 (90.32%) were culture positive while 3 (9.68%) had no growth. Fourty four isolates were obtained, with *Staphyloccocus aureus* 18 (40.9%), *Proteus spp* 13 (29.5%), *Pseudomonas aeruginosa* 8 (18.2%), *Klebsiella spp* 4 (9.0%) and *Escherichia coli* 1 (2.4%). There were 10 (32.5%) mixed infections. *Klebsiella spp*, *Proteus spp* and *pseudomonas aeruginosa* were fully susceptible to ciprofloxacin and ceftazidime. The *E.coli* isolate was cipofloxacin resistant and *S.aureus* showed multidrug resistance.
Conclusion: CSOM is more prevalent in seropositive children. Resistance to commonly used antibiotics was high.

3.407
Bacterial causes of pneumonia in children under 5 years of age in Nairobi, Kenya.
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Background: Pneumonia kills more children than any other illness and is a significant problem worldwide.
Objective: To determine spectrum and antibiogram of bacterial causes of mild and severe pneumonia in children under five years.
Design: Cross sectional laboratory based.
Setting: Kenyatta National hospital (KNH) and Mbagathi District hospital (MDH) in Nairobi, Kenya.
Subjects: Children under 5 years presenting with clinical pneumonia between 2002 to 2005.
Methods: Bacterial culture of nasopharyngeal aspirate (NPA) and 5 ml venous blood was done on blood agar, chocolate agar, bromothymol blue and brain heart infusion broth. Identification performed using analytical profile index (API) and drug susceptibility using agar dilution as recommended by clinical and laboratory standards institute (CLSI).
Results: A total of 639 respondents were investigated for bacterial causes of mild (459) and severe (180) pneumonia. The bacterial spectrum in mild and severe pneumonia was different with the first two being S. *pneumonia* 98/210 (46.67%) and *H. influenza* 20/210 (9.52%) in mld and *K. pneumonia* 10.0% and *S. aureus* 7.62% in severe pneumonia
Penicillin resistant *S. pneumonia* was 4.6% with 37.9% intermediate resistance. Trimethoprim/sulfamethoxazole (TMP-SMX) and gentamicin was 83.8% and 34.8% respectively. There was high level resistance exhibited by *K. pneumonia* and *S. aureus* to he commonly used antibiotics for treatment of pneumonia.
Conclusion: The spectrum of bacterial causes of mild and severe pneumonia is different with *S. pneumonia* and *K. pneumonia* being the major causes respectively. Penicillin is still the drug of choice for treatment of pneumococcal pneumonia. More drug resistance ssurveillance studies are encouraged.

3.407

Pneumococcal Conjugate Vaccine at birth provokes effective IgG titres and primes immunological memory

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Background. In Kenya, 15% of invasive pneumococcal disease (IPD) targeted by new Pneumococcal Conjugate Vaccines (PCV) occurs before young infants are routinely immunised. Newborn immunisation could protect young infants but there are theoretical concerns it could lead to immune tolerance.
Methods. In a randomised schedule trial children received either 7-valent PCV at 6-10-14 weeks (EPI group) or 0-10-14 weeks (Newborn group). Safety was monitored actively, at 2 or 7 days, and passively thereafter. Serum was obtained at birth, 6, 10, 14, 18, 36 and 37 weeks and assayed by ELISA for concentration and avidity of anti-capsular IgG. Infants were boosted with either 7vPCV or one-fifth dose of pneumococcal polysaccharide vaccine at 36 weeks. Nasopharyngeal swabs were taken at 18 and 36 weeks.
Results. Three-hundred children were randomised equally into the vaccine groups. Newborn vaccination was well tolerated and safe; adverse events occurred in equal frequency in each group and none was related to immunisation. One child, immunised at birth, died of unrelated neonatal sepsis. At 18 weeks ≥87% of infants had protective concentrations (≥0.35 µg/ml) for all serotypes with no significant differences between groups. Geometric mean concentrations were higher in the EPI group for serotypes 4, 9V, 18C and 19F at 18 weeks and serotype 4 at 36 weeks. Avidity was greater in the Newborn group for serotypes 4, 6B and 19F at 18 weeks and

serotype 19F at 36 weeks. Booster responses and the prevalence of vaccine-serotype and non-vaccine-type carriage did not differ between groups.
Conclusions. PCV was safe, immunogenic and primed for memory when given shortly after birth. There was no evidence of immune tolerance. Where IPD occurs early, vaccination coverage is delayed or indirect protection fails, newborn vaccination offers an alternative to control IPD in vulnerable young infants.

3.408

Routine Cryptococcal Screening and Treatment in Nyanza Province: Preliminary Outcomes

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Background: Cryptococcal meningitis (CM) is a leading cause of mortality in HIV-infected individuals. Screening patients with low CD4 cell counts for serum cryptococcal antigen (sCrAg) may identify those at risk for developing CM. Our objective is to describe the preliminary outcomes of HIV-infected individuals with CD4≤100 and positive sCrAg.
Methods: Family AIDS Care and Education Services (FACES) began a screening and treatment intervention in Nyanza province in which sCrAg was tested in newly enrolled patients with CD4≤100. An individual was considered sCrAg positive if titer was ≥1:2. Patients with positive sCrAg were treated with high-dose fluconazole. Patient files from November 2009 through February 2010 for all individuals with positive sCrAg were reviewed 6 months after enrollment for demographics, symptoms, treatment and outcomes. Cox proportional hazards regression was used to explore associations between baseline characteristics and survival.
Results: Of newly enrolled individuals with CD4≤100 (n=600), 47 (8%) were sCrAg positive. Among sCrAg positive individuals, the mean age was 34.8 years, 53% were male, and the mean CD4 cell count was 34. There were 14 deaths (30%) during the total follow-up time of 963 patient-weeks. Median survival was 12 weeks [95%CI: 3, 17]. In bivariate analysis, receiving antiretroviral therapy (ART) (HR=0.10, p<0.01) and fluconazole (HR: 0.30, p=0.03) were significantly associated with survival. Gender, age, and baseline CD4 count were not significantly associated with survival. In multivariate models, only ART remained significantly associated with survival (HR=0.10, p<0.01). Median survival among individuals who did not receive ART or fluconazole was 3 weeks.
Conclusion: Patients with low CD4 who are sCrAg positive and received ART have higher survival rates. Patients not receiving ART or fluconazole have higher mortality and die soon after enrolling in care. However, these individuals may not have survived long enough to initiate ART or fluconazole. Further analysis is planned.

3.409

High cost of unreliable Widal test in diagnosis of Typhoid fever among patients attending Alupe Sub district Hospital

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Background: Typhoid fever has remained a global health problem. Annually, the WHO estimates 22 million new cases and 5% fatal cases. The current diagnosis of *Salmonella* uses the Widal test. However, has a low

specificity. Therefore, there is irrational use of antibiotics, leading to unnecessary expenditure of income.

Objective: To analyze unnecessary expenditure incurred by non specific results of widal test in the diagnosis of typhoid fever.

Methodology: This was 3 year cross-sectional study between 2007 and 2009. 18,866 outpatients with signs and symptoms of Typhoid fever at Alupe sub-district hospital were recruited. Blood sample was taken and a widal test done. Those found positive were treated with Augumentin, ciprofloxacin and Chloramphenicol.

Results: Out of the total (18,866) patients, 2264(12%) had widal test positive. These were 403(17.8%) children <12 years and patients ≥12 years; 1861(82.2%). 356 and 47 children <12 years were given Augumentin and ciprofloxacin respectively. 1070 and 461 patients ≥12 years were given Chloramphenicol and Ciprofloxacin respectively. Augumentin costed 200/= per dose/child <12 years. Ciprofloxacin costed 40/= per dose/child <12 years and 80/= per dose/patient ≥12 years. Chloramphenicol costed 224/= per dose/patient ≥12 years. The total amount spend was 423,560/= equivalent to 5,648U$.

Discussion: In reference to studies done by Kariuki *et al.*200**,** 100% widal test positive, only 5% are true by gold standard culture. Therefore, 2264 patients who had widal test positive, only 113 might have had the bacteria, the remaining 2151 might not have harbored the bacteria and yet were put on treatment. This might have resulted to unnecessary additional expenditure of 402,420/= instead of 21,200/=.

Conclusion: An alternative cheaper, sensitive and specific novel Diagnostic technique need to be developed to offer a paradigm shift in the way *Salmonella Typhi* is being detected. This will reduce the threat posed by antibiotic abuse and unnecessary expenditure.