MULTI-PEPTIDE ELISAS OVERCOME CROSS-REACTIVITY AND INADEQUATE SENSITIVITY OF CHLAMYDIA TRACHOMATIS AND C. PNEUMONIAE SEROLOGY

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Background Chlamydia spp. serology is compromised by cross-reactivity of classical antigens. For specific detection of anti-trachomatis (Ctr) and anti-C. pneumoniae (Cpn) antibodies, we developed and validated novel peptide ELISAs.

Methods Strongly reactive peptide antigens of 24 Ctr- and 48 Cpn-specific B-cell epitopes of multiple immunodominant chlamydial proteins were used in this study. For specific detection of anti-Ctr and anti-Cpn antibodies, 185 human sera were tested in colorimetric ELISAs with mixtures of 12–24 Ctr or Cpn peptide antigens using polyclonal anti-human IgG-HRP conjugates. For comparative evaluation, these sera were tested with 4 Ctr and 4 Cpn commercial IgG ELISAs.

Results In commercial ELISAs, Ctr and Cpn individual serum reactivity was 54% biased towards positivity for both species (co-positivity), but unbiased in Ctr and Cpn peptide antibody assays. This finding suggested a severe specificity problem (cross-reactivity) of commercial ELISAs, but not peptide assays. Using hyperimmune mouse sera against each of 11 Chlamydia spp., we confirmed that commercial Ctr and Cpn ELISA antigens are cross-reactive among all Chlamydia spp., but Cpn and Ctr peptide antigens react specifically only with antisera against the cognate chlamydial species. By comparison at 90% specificity to a Ctr-peptide composite reference standard (CRS) for human anti-Ctr antibody status, the Ctr mixed peptide assays showed 86–83% sensitivity, significantly higher than the 59–34% sensitivity of 4 commercial anti-Ctr ELISAs. Relative to a Cpn-peptide CRS, the Cpn mixed peptide assay showed 86–80% sensitivity at 90% specificity, significantly higher than the 48–25% sensitivity of 4 commercial anti-Cpn ELISAs.

Conclusion For detection of anti-Ctr and -Cpn antibodies, commercial ELISAs are not suitable due to cross-reactivity. In contrast, mixed peptide assays are accurate with simultaneous high specificity and sensitivity, and reliably determine anti-Ctr and anti-Cpn antibody prevalence. With convenient use for non-specialized laboratories, these peptide ELISAs will improve Ctr and Cpn serodiagnosis.

Disclosure No significant relationships.

PENELE MICROBIOME AND URINARY CYTOKINES OF KENYAN MEN WHO HAVE SEX WITH MEN AND MEN WHO HAVE SEX WITH WOMEN

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Background MSM are disproportionately affected by HIV and STIs compared to men who have sex with women (MSWomen). This may be due in part to different burden of mucosal inflammation. We compared penile microbial composition between MSM and MSWomen and association with mucosal inflammation.

Methods In this cross-sectional study, we enrolled 43 MSM and 43 MSWomen, who were HIV negative and matched on age and circumcision status. The penile microbiome was assessed via metatal swab, with 16S rRNA gene amplicon sequencing. Urinary cytokine concentrations (TNF-a/IL-1b/IL-8/IL-10/IP-10) were measured using Luminox. Random Forest (RF) identified genus-level taxa differing between MSM and MSWomen. Taxa from RF were regressed on cytokine outcomes, with multiple testing correction and information criterion model selection.

Results Men were median age 24 and 77% circumcised. There were substantial differences in educational attainment, employment, alcohol and drug use, condom use, and number of sexual partners, with MSM having greater behavioral risks. Microbiome composition differed markedly between MSM and MSWomen: RF discriminated between MSM and MSWomen with 84% accuracy. Taxa with greatest
discriminating influence were *Lactobacillus*, *Anaerococcus*, and *Staphylococcus*. In crude analysis, cytokines TNF-α/IP-10/IL-10 were elevated among MSWomen (p<0.05, each); IL-8 did not differ by group; IL-1β was higher among MSM (p=0.03). Cytokine concentration increased in response to Corynebacterium (IL-8/TNF-α/IP-10/IL-1β), Gardnerella (IL-8/IP-10/IL-1β), Veillonella (IL-8/IP-10/IL-1β), and Peptoniphilus (IL-8/IL-1β). Microbiome composition did not account for the difference in TNF-α, IP-10, or IL-10 between groups; the difference in IL-1β became non-significant after accounting for taxa. Among MSWomen, IL-1β (p=0.01) and IL-8 (p=0.05) were elevated if the female partner had BV.

**Conclusion** To our knowledge, this is the first comparison between MSM and MSWomen of penile microbiome and urinary cytokines. Future studies should examine whether microbiome and mucosal inflammation differences between MSM and MSWomen cause differential risk of HIV/STI acquisition or differential impact on efficacy of HIV/STI interventions.

**Disclosure** No significant relationships.

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**P858** 2018/2019 SURVEILLANCE UPDATE ON NEISSERIA GONORRHOEAE ISOLATES

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**Background** The global prevalence of antimicrobial resistance (AMR) in Neisseria gonorrhoea (GC) is increasing and of specific concern is the emerging resistance to third generation cephalosporins worldwide. In Africa, exceedingly limited AMR data is available. The study determined the AMR in GC isolates a public referral clinics offering HIV and STI testing and treatment to people living in Nairobi and the region.

**Methods** The survey on men presenting with urethral discharge at the special treatment Clinic (STC-Casino Clinic) collected samples from symptomatic men, inoculated on modified Thayer martin media (MTM) and identified by standard bacteriological methods. The MICs of five antibiotics Azithromycin, Gentamycin, ciprofloxacin, ceftriaxone and cefixime were determined by the Etest method (AB Biodisk, Solna, Sweden) and results defined as susceptible, intermediate and resistant. WHO reference strains were used as controls.

**Results** A total of 153 samples have been collected with 96 samples having tested culture positive, giving a 62.7% prevalence on samples collected from 25th June 2018 to 5th February, 2019. The mean MIC of 0.016 was recorded for Azithromycin, cefixime, while a mean MIC of 1.41 and 2.0 was recorded for Ciprofloxacin and Gentamycin respectively. The MIC range for Ciprofloxacin and Gentamycin was from 0.004 to 6 and from 0.125 to 8 respectively.

**Conclusion** This is a continuous study on the Gonococcal surveillance program to describe antimicrobial resistance profiles of antibiotics used in the region. It confirms that N. gonorrhoea isolates from Nairobi in 2018 possessed high level resistance to Ciprofloxacin as antimicrobials previously recommended for the treatment of gonorrhea. Cefixime, ceftaxone and azithromycin are still useful drugs for treatment of gonococcal infections in Kenya. The outcome of this study together with other additional studies will enable revisions of the gonorrhea treatment guidelines in Kenya and support in antimicrobial resistance in the region.

**Disclosure** No significant relationships.

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**P859** GENOTYPING gyrA AND penA FROM REMNANT NEISSERIA GONORRHOEAE POSITIVE CEPHEID XPERT® CLINICAL SPECIMENS

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**Background** Neisseria gonorrhoeae (NG) has developed resistance to most antibiotics, making it increasingly difficult to treat. Molecular methods have been used to predict antimicrobial susceptibility based on the gyrA codon serine 91 and the mosaic XXXIV allele on the penicillin-binding protein 2 (penA) gene using Roche Cobas and APTIMA clinical specimens. We aimed to determine if the same methods could be successfully used on remnant NG-positive Cepheid Xpert® specimens.

**Methods** We tested NG-positive pharyngeal, rectal, and vaginal/urine specimens from adolescents aged 14–24 years. We extracted 100uL DNA from each sample using the Roche® MagNA Pure. The Roche LightCycler® 480 was used to genotyped gyrA and penA in a multiplex PCR using high resolution melt curve analysis. The fluorescent labels of the detection probes for the penA mosaic XXXIV target (Cyanine-5 dye) differed from that of gyrA (LightCycler® 640 probe) so that both genes could be detected simultaneously at various wavelengths. We used isolates with previously confirmed presence of the NG mutant gyrA, NG wild type gyrA, and mosaic penA XXXIV allele for internal controls.

**Results** Of the clinical specimens, 62% (38/61) were successfully genotyped. Urine specimens were most likely to be genotyped (5/6, 83%) followed by rectal (19/26, 73%), pharyngeal (12/24, 50%), and vaginal specimens (2/5, 40%). Of the 38 genotyped specimen, 8 had the penA XXXIV allele (2/26 rectal, 6/24 pharyngeal) and 16 had a mutated gyrA (10/26 rectal, 3/24 pharyngeal, 2/6 urethral, 1/5 vaginal). Of the 8 penA XXXIV positive specimens, 6 were gyrA indeterminate, 1 was gyrA wild type, and 1 was gyrA mutant. Of the 30 specimens without the penA XXXIV mosaic allele, 15 were gyrA wild type and 15 were gyrA mutant.

**Conclusion** Genotyping specific NG genes from Cepheid Xpert® clinical specimens was feasible. Our study was limited by its small sample size and lack of concurrent antimicrobial testing.

**Disclosure** No significant relationships.

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**P861** NOVEL MUTATION CONFOHRING HIGH-LEVEL AZITHROMYCIN RESISTANCE IN NEISSERIA GONORRHOEAE

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**Background** Azithromycin resistance in Neisseria gonorrhoeae has been attributed to several resistance-associated mutations