

**P1.09** **DIAGNOSING *MYCOPLASMA GENITALIUM*: COMPARISON OF FOUR NUCLEIC ACID AMPLIFICATION TESTS AND USE OF THE SPEEDX REAL-TIME PCR ASSAY FOR AZITHROMYCIN RESISTANCE**

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**Introduction** *Mycoplasma genitalium* (MG) is a sexually transmitted infection (STI) associated with urethritis in men and cervicitis and pelvic inflammatory disease (PID) in women. Azithromycin (AZ) resistance in MG, resulting in treatment failure is an issue. We compared molecular assays for detection of MG in females; a prospective cohort of adolescents with PID and a retrospective group of women at a STD clinic. We tested vaginal samples for AZ resistance with the SpeedX assay, a real-time PCR, which detects MG and 23S rRNA AZ resistance mutations.

**Methods** For the prospective study, SpeedX results were compared to 16S rRNA and pdhD real-time PCR assays, and the Aptima MG ASR assay. Samples consisted of dry transported vaginal swab samples (n=116); expressed in water. For the retrospective study we evaluated vaginal samples (n=289) stored in liquid Amies media. Nucleic acid extraction was performed utilising the Roche MagNA Pure LC robot according to manufacturer instructions. The SpeedX assay was performed according to manufacturer instructions. For the retrospective study, results were compared to the 2 real-time PCR assays for the 16S rRNA gene and pdhD gene.

**Results** For the prospective study, a gold standard of 3 of 4 positive tests defined a true positive. The 16S PCR had 100% sensitivity (10/10) and 100% specificity (106/106) Kappa=1 [95% CI: 1–1], while SpeedX PCR displayed 90% sensitivity (9/10) and 99% specificity (105/106) Kappa=0.89 [95% CI: 0.74–1]. The pdhD PCR had 100% sensitivity (10/10) and 100% specificity (106/106) Kappa=1. The Aptima MG assay had 90% sensitivity (9/10) and 94% specificity (100/106) Kappa=0.69 [0.46–0.91]. The SpeedX 23S rRNA mutation rate was 50% (5/10). For the retrospective group, no Aptima MG was done because of transport media. A gold standard of 2 of 3 positive tests defined a true positive. The 16S PCR was 84% sensitive (21/25) and 100% specific (264/264) Kappa=0.91 [0.813–0.997]. The SpeedX was 92% sensitive (23/25) and 99% specificity (262/264) Kappa=0.91 [0.833–0.997]. The pdhD PCR was 96% sensitive (24/25) and 99% specific (261/264) Kappa=0.91 [0.833–0.997]. The SpeedX AZ resistance was 36% (9/25).

**Conclusion:** More prospective evaluation studies are required; all assays performed well. The SpeedX PCR compared well to two real-time PCRs and the Aptima MG assay. High AZ resistance was observed. The SpeedX assay is potentially useful for MG diagnosis and for detection of resistance to AZ.

**P1.10** **ORAL FLUID BASED RAPID SYPHILIS TESTING**

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**Introduction** Point-of-care syphilis testing currently uses finger-stick blood to identify *Treponema pallidum* (TP) antibodies by

qualitative immunoassay. However, oral fluid testing is highly preferred by patients. We aimed to determine if rapid tests intended for whole blood could be used to detect treponemal antibody in oral fluid.

**Methods** Oral fluid was collected at the UCLA Care Clinic from 49 participants using Oasis SuperSAL (Vancouver, WA). The device uses an absorbent cylindrical pad to collect and filter ~1 mL of oral fluid. Oral fluid filtrate was evaluated with 3 rapid syphilis tests: SD Bioline Syphilis 3.0 (SD), Med-Mira Reveal TP (MM), and First Response Anti-TP (FR) following manufacturer directions for whole blood. Reference test results, TP particle agglutination (TPPA) and rapid plasma reagin (RPR), were extracted from participants' medical records. We used 3 different definitions as a reference standard. Definition 1: TPPA reactive. Definition 2: TPPA and RPR reactive. Definition 3: TPPA reactive and RPR titer >1:4. Specimens nonreactive on both the TPPA and RPR were considered negative. We calculated specificity and sensitivity for each definition, and used the exact binomial method to determine 95% confidence intervals (CI).

**Results** With definitions 1, 2 and 3 respectively, SD sensitivity was 86.4% (CI: 65.1, 97.1), 93% (CI: 66.1,99.8), 100% (CI: 59.0,100); MM sensitivity was 6.3% (CI: 0.2,30.2), 9.1% (CI: 0.2,41.2), 16.7% (CI: 0.4,64.1); and FR sensitivity was 25% (CI: 8.7,49.1),25% (CI: 5.5,57.2), 14.3% (CI: 0.4,57.9). SD specificity was 56% (CI: 36.5,75.5), MM specificity was 100% (CI: 35.9,99.6) and FR specificity was 100% (CI: 83.9,100).

**Conclusion** The high sensitivity of the SD test suggests a strong potential for oral fluid-based rapid syphilis testing. SD sensitivity increased with RPR titer. False positive results may be associated with the presence of non-venereal treponemal antibodies in oral fluid. The MM and FR tests had low sensitivity, perhaps due to differences in treponemal targets. Further research and development are needed.

**P1.11** **DAMPENING OF THE MTRCDE EFFLUX PUMP OPERON IN *NEISSERIA GONORRHOAE* STRAIN HO41 ENHANCES GONOCOCCAL SUSCEPTIBILITY TO PENICILLIN**

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**Introduction** The resistance to extended-spectrum cephalosporins (ESCs), which is the only remaining antibiotic in many regions for treatment of gonorrhoea, expressed by certain strains of *Neisseria gonorrhoeae* is a major public concern worldwide. The first “superbug” reported in Japan was referred to HO41, which was found to have high level resistance to ESCs and most other available antibiotics. Non-beta-lactamase resistance of gonococci to beta-lactam antibiotics requires several chromosomal mutations including a promoter mutation (single bp deletion in the mtrR promoter) that results in over-expression of the MtrCDE efflux. This promoter mutation results in loss of production of MtrR, which is the transcriptional repressor of *mtrCDE*, and is present in HO41. Here we investigated the possibility of reverting the resistant HO41 to be susceptible to  $\beta$ -lactam antibiotics by expressing MtrR and dampening the MtrCDE efflux pump.

**Methods** MtrR was ectopically expressed in HO41 (named as SC4) and confirmed to be functional by western blot and qRT-PCR analyses. HO41 and SC4 were compared for their susceptibility to antibiotics in laboratory media and in the presence of ME180 cervical epithelial with or without IPTG induction.

**Results** In both laboratory media and in ME180 cell culture, we found that expression of MtrR in SC4 (HO41 *mtrR+*) decreased *mtrCDE* gene expression and increased gonococcal susceptibility to beta-lactam antibiotics. Importantly, MtrR-mediated repression of *mtrCDE* decreased the MIC of penicillin to a level below the MIC breakpoint recommended clinical treatment dose.

**Conclusion** We demonstrate the MtrR-mediated dampening of *mtrCDE* can greatly increase gonococcal susceptibility to penicillin. Thus, novel adjunctive therapeutics that decrease levels of MtrCDE may allow for the return of penicillin as an option for treating otherwise resistant strains of gonococci.

**P1.12** **ROLE OF VAGINAL LACTOBACILLI IN COUNTERACTING CHLAMYDIA TRACHOMATIS INFECTIVITY IN AN IN VITRO MODEL**

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**Introduction** Lactobacilli play a fundamental role in maintaining the ecological equilibrium of the vaginal niche, preventing the overgrowth of endogenous microorganisms and impeding the colonisation of pathogens. Although many studies have focused on the mechanisms displayed by lactobacilli in counteracting several urogenital pathogens, a few data are available on the interaction between lactobacilli and *Chlamydia trachomatis* (CT). The aim of this study was therefore to assess the *in vitro* activity of different vaginal *Lactobacillus* strains against CT infectivity, investigating two different fractions of bacteria (cell pellets and cell-free supernatants), by three different mechanisms of action (competition, exclusion and displacement).

**Methods** A total of 17 *Lactobacillus* strains, isolated from vaginal swabs of healthy premenopausal women and belonging to *L. crispatus* (BC1; BC3-BC8), *L. gasseri* (BC9-BC14), *L. vaginalis* (BC16-BC17) and *L. plantarum* (BC18-19) species, were included in the study. The capacity of lactobacilli cell pellets (CP) and cell-free supernatants (CFS) to interfere with CT adhesion and entry in HeLa epithelial cells was evaluated, by means of competition, exclusion and displacement mechanisms. In particular, lactobacilli fractions corresponding to  $5 \times 10^7$  colony forming units (CFU) were incubated with  $5 \times 10^3$  CT elementary bodies (EBs) of strain GO/86, (serotype D), following different timelines. CT infection was evaluated by counting chlamydia inclusion forming units (IFUs) by direct immunofluorescence. Moreover, on the basis of CT infectivity interference results, 5 lactobacilli were selected for dose-effect assays and the same experiments were repeated, using CP or CFS fractions with  $5 \times 10^6$  and  $5 \times 10^5$  lactobacilli cells, in order to verify if a dose-dependent activity was present. Finally, the capacity of *Lactobacillus* strains to adhere to HeLa cells was assessed as well: results were read at light-microscopy and HeLa cells were scored for the presence and number of lactobacilli attached.

**Results** We found that lactobacilli cell pellets were the most active fraction in counteracting CT infectivity, particularly by means of an exclusion strategy, and that *L. crispatus* was the most effective species, even though a strain-specific activity was detected. Moreover, the anti-chlamydial activity was not correlated with the level of lactobacilli adhesion on epithelial cells and it was significantly maintained with low numbers of lactobacilli, although in presence of a dose-response effect.

**Conclusion** We identified specific vaginal *Lactobacillus* strains (*L. crispatus* BC4, *L. crispatus* BC5, *L. crispatus* BC7, *L. gasseri* BC14 and *L. plantarum* BC19) able to interfere with CT EBs adhesion and entry in epithelial cells and we were able to shed light on the mechanisms displayed by lactobacilli in counteracting CT infectivity. A major potential application lies on the use of these *Lactobacillus* strains as probiotics for the prophylaxis and/or adjuvant therapy of CT infections.

**P1.13** **VAGINAL MICROBIOME SIGNATURES IN CHLAMYDIA TRACHOMATIS INFECTED WOMEN**

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**Introduction** In healthy women, lactobacilli play a crucial role in maintaining the microbial homeostasis of the vaginal niche. In case of bacterial vaginosis (BV), a condition characterised by a depletion of lactobacilli and an increasing number of anaerobes, a higher risk of urogenital and sexually transmitted infections (STIs) is reported. The vaginal environment of healthy and BV-positive women have been extensively studied, leading to the identification of the microbial species dominating these opposite conditions and to the description of specific metabolic profiles. Besides that, less is known about the vaginal microbiome in case of STIs, as *Chlamydia trachomatis* (CT) infections. The aim of this study was to analyse the composition of the endogenous microbiota and the metabolic signatures of the vaginal niche in 3 different conditions: healthy, BV and CT infections.

**Methods** From July 2016, all the pre-menopausal women attending the STI Outpatients Clinic of Sant'Orsola-Malpighi Hospital in Bologna (Italy) and meeting one of the following criteria were enrolled: presence of vaginal symptoms or presence of risk factors for CT infection. Patients with vaginal candidiasis were excluded. For all the patients, a vaginal swab was collected for molecular CT detection (Versant CT/GC DNA 1.0 Assay; Siemens), whereas Amsel criteria were used for BV assessment. Moreover, for each woman, an additional vaginal swab stored in saline was collected and centrifuged. Cell pellets were examined with a DNA-microarray platform including 17 probe sets specific for the most representative vaginal bacterial groups and with a quantitative real-time PCR targeting 16s rRNA gene of *Gardnerella vaginalis* (GV). Cell-free supernatants were used for metabolomic analysis by means of <sup>1</sup>H-NMR spectroscopy. NMR spectra were recorded with an AVANCE spectrometer (Bruker). Similarities among microbial and metabolic profiles of samples were investigated by means of a principal component analysis (PCA). Differences in GV DNA loads and metabolites concentrations were