P1.26 EVALUATION OF TWO DIAGNOSTIC SYSTEMS FOR THE DETECTION OF *CHLAMYDIA TRACHOMATIS* (CT) AND *NEISSERIA GONORRHOEAE* (NG) USING MALE URETHRAL SWABS

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Introduction Urine is the specimen of choice for CT and NG molecular testing from males and many of the next generation testing platforms are not FDA approved for use with urethral swabs. Urethral swabs remain valuable in research settings that may require that the specimen is collected in a manner preserving organism viability. The goal of this study was to assess the Abbott *m*2000 Realtime (*m*2000), and Roche COBAS 4800 (c4800) systems for the identification of CT and NG in urethral swabs collected in chlamydia transport medium (CTM).

Methods Archived male urethral swabs collected in CTM from STD clinic attendees were tested. These specimens were originally tested for CT and NG on a platform FDA-approved for urethral swabs collected in CTM (Roche COBAS Amplicor, (cAMP)).Two-hundred µl of CTM was added into each manufacturer's collection device. This was subsequently tested on the *m*2000 and c4800 systems according to the manufacturer's instructions. CT and NG results obtained from each platform were compared to the original cAMP results to determine sensitivity and specificity. Agreement between platforms was measured by calculating the kappa coefficient.

Results One hundred urethral swab specimens were available for testing (28 CT and 25 NG positives). When compared to results obtained with the cAMP, the sensitivity and specificity of the *m*2000 for the detection of CT was 100% and 98.6%, respectively; the sensitivity and specificity on the c4800 was 96.4% and 98.6%. For NG, the sensitivity and specificity of the *m*2000 assay was 100% and 98.7%; the c4800 assay was 100% sensitive and specific. Agreement was excellent for both platforms when compared to cAMP with kappa scores of >0.95 for CT and >0.97 for NG.

Conclusion The *m*2000 and c4800 platforms have similar performance characteristics to cAMP for the detection of CT and NG using urethral swabs in CTM. This study will provide investigators with additional options when designing protocols that require the preservation and ability to recover viable organisms from men.

P1.27 HPV IS ASSOCIATED WITH AN ALTERED METABOLOMIC PROFILE IN THE VAGINAL TRACT

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Introduction Recent studies have reported associations between bacterial vaginosis (BV) and cervicovaginal HPV. To obtain further insight into this relationship, we examined the vaginal microbiota and metabolome of women who were HPV+ and HPV-.

Methods Thirty-nine women self-collected mid-vaginal swabs that were profiled for bacterial composition by 16S rRNA gene amplicon sequencing, metabolites by both GC/MS and LC/MS-based metabolomics and 37 types of HPV DNA with the Roche HPV Linear Array genotyping test. Data were analysed by multiple linear regression controlling for confounding factors, as well as principal components analysis, partial least squares discriminatory analysis and linear discriminant analyses. All reported results have an adjusted p-value (q-value) <0.05.

Results Vaginal microbiota were clustered into community state types CST-I (*L. crispatus*-dominated), CST-III (*L. iners*-dominated) and CST-IV (low-*Lactobacillus*/BV-associated taxa). Overall, HPV+ women had higher polyamine and phospholipid concentrations than HPV- women in a model which controlled for CST and smoking status. Significant differences in metabolomic profiles of HPV+ and HPV- women were also evident in each stratum of CST. Among women who were CST-III, HPV+ women had higher concentrations of biogenic amines and glycogen-related metabolites compared to HPV- women. Within CST-IV, HPV+ women had lower concentrations of glutathione, glycogen, and phospholipid-related metabolites than HPV- women. Women with high-risk HPV strains had lower concentrations of amino acids, lipids and peptides compared to women who were hrHPV-.

Conclusion Detection of HPV was associated with altered vaginal concentrations of biogenic amines, glutathione and lipid-related metabolites. Reduced glutathione and oxidised glutathione have known associations with HPV and may be representative of increased oxidative stress and total glutathione depletion. Elucidating a causal relationship between microbial metabolites associated with increased oxidative stress and HPV infection warrants further investigation.

P1.28 PRODUCTION OF A POLYCLONAL ANTIBODY AGAINST THE VAGINOLYSIN OF *GARDNERELLA VAGINALIS*

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Introduction Bacterial vaginosis (BV) is a polymicrobial syndrome characterised by the decrease in Lactobacilli and an increase of anaerobic bacteria, mainly *G. vaginalis* a Gram-positive coccobacillus that is isolated in up to 98% of BV cases. This bacteria produces differents virulence factors like sialidase, succinate, biofilm formation, phospholipase C, and vaginolysin (VLY). The VLY is a protein of 56 kDa that belongs to the family of cholesterol-dependent cytolysins, the function of this protein is the cellular lysis of erythrocytes and epithelial cells through the binding to the CD59 receptor and cholesterol present in cell membranes. The production of a policlonal antibody agains VLY will allow the study of this cytolysin in the pathogenesis of *Gardnerella vaginalis* in the vaginal tract.

Methods We use as antigen the ATCC 14018 of *G. vaginalis* wich was obtained from a extract of total proteins by electrophoresis. The band corresponding to the molecular weight of the VLY (56 kDa) was cut, macerated and resuspended in PBS. Two New Zealand rabbits were immunised for 6 weeks,

for the first immunisation Freund's complete adjuvant was used while for the second to fifth immunisation we use Freund's incomplete adjuvant, in the sixth week only the antigen was used. At week 0, 3 and 6, rabbits were bled for the evaluation of the immune response by the indirect ELISA. The final bleeding was performed and the serum obtained was stored at −20°C until use. For he purification of the antibodies we use a protein A purification kit and the integrity of the immunoglobulins was verified by electrophoresis, the titration of the obtained polyclonal antibody was performed by indirect ELISA. We evaluated the antibody by Western blot, Dot blot and inhibition of hemagglutination using as antigen the ATCC 14018 of *G. vaginalis*.

Results The first evaluation of the immune response of rabbits showed that prior to immunisation the rabbits had no antibodies against VLY, whereas during the third and sixth week of immunisation they had antibody titres of 1:250 and 1:1000 respectively. Electrophoresis of the polyclonal antibody showed the purity and integrity of the purified antibodies, this antibodies can be use at titers of 1:1000 for the subsequent assays. The western blot showed that this antibody recognised a band of approximately 56 kDa that matched with the molecular weight reported for the VLY, whereas the Dot blot showed that the antibody recognises the VLY of ATCC 14018 of *G. vaginalis*. To corroborate that the antibody inhibited erythrocyte lysis, hemagglutination inhibition assays were performed and we showed that the use of this antibody decreased the cell lysis in around 80%.

Conclusion We produce a polyclonal antibody agains the VLY of *G. vaginalis* capable of inhibiting the erythrocyte lysis. This antibody will be useful in investigating the role of VLY in the pathogenesis of *G. vaginalis* during the development of BV.

P1.29 ATTACHMENT OF THE SYPHILIS SPIROCHETE, *TREPONEMA PALLIDUM*, TO THE VASCULAR ENDOTHELIUM

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Introduction: *Treponema pallidum* is the causative agent of venereal syphilis, a human-specific sexually transmitted infection characterised by multi-stage disease and diverse clinical manifestations. *T. pallidum* undergoes rapid hematogenous dissemination, accessing distant organ sites and penetrating tissue, placental, and blood-brain barriers. Tp0751 is an adhesin that interacts with the host vasculature and mediates bacterial adherence to endothelial cells under shear flow conditions. This study explores Tp0751-mediated adhesion to the vascular endothelium.

Methods Tp0751, expressed in a non-infectious model spirochete [*Borrelia burgdorferi* (*Bb*-Tp0751)], was assessed for a gain-of-function adhesion phenotype using attachment assays. Interaction specificity was probed with competitive inhibition studies using synthetic peptides of Tp0751 host-binding regions. Affinity chromatography coupled with mass spectrometry was used to identify endothelial receptors for Tp0751. Membrane receptors isolated from human umbilical vein endothelial cells (HUVECs) were incubated with Tp0751-affinity columns and interacting proteins were identified with mass spectrometry.

Results Here we demonstrate that *Bb*-Tp0751 adheres to HUVECs under stationary conditions. The laminin receptor (LamR) was identified as an endothelial receptor for Tp0751. LamR is a brain endothelial receptor for other neurotropic invasive pathogens, including *Neisseria meningitidis*. Current investigations will validate the Tp0751-LamR interaction and characterise the functional outcomes of Tp0751 adhesion to endothelial cells.

Conclusion These investigations reveal the mechanics of *T. pallium* attachment to endothelial cells, the fundamental step in the process of *T. pallidum* vascular dissemination. A complete understanding of this process will provide opportunities to prevent *T. pallidum* attachment to the host vasculature to facilitate syphilis vaccine development.

P1.30 IN VITRO ACTIVITY OF GEPOTIDACIN AND OTHER ANTIMICROBIALS AGAINST MYCOPLASMAS AND UREAPLASMAS

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Introduction *Mycoplasma* and *Ureaplasma* spp. are important pathogens of the respiratory and urogenital tracts. Antimicrobial resistance limits treatment options. Gepotidacin (GEP), a novel triazaacenaphthylene topoisomerase II inhibitor that inhibits DNA replication by a mechanism and target distinct from fluoroquinolones was tested against 85 isolates of *Mycoplasma pneumoniae* (Mp), *Mycoplasma hominis* (Mh), *Mycoplasma genitalium* (Mg), *Ureaplasma parvum* (Up), and *Ureaplasma urealyticum* (Uu) in comparison to azithromycin (AZI), clindamycin (CLI), tetracycline (TET), levofloxacin (LEV), and moxifloxacin (MOX). Organisms tested included strains known to be resistant to TET, LEV, and/or AZI. This work was supported by GSK and funded through OTA HHSO100201300011C with HHS/BARDA.

Methods MICs were determined using broth microdilution in accordance with Clinical and Laboratory Standards Institute Guidelines.

Results GEP was active against 25 Mp, MIC range 0.032–0.125 µg/ml, including 5 that were AZI-resistant, with MIC_{90} (0.125 µg/ml), equivalent to MOX. GEP was active against 10 Mg. MIC_{90} (0.032 µg/ml) was 4-fold < MOX. GEP MICs against 25 Mh ranged from 0.5 to 2 µg/ml with MIC_{90}=2 µg/ml, making it less active than other agents, including MOX (MIC_{90}=0.125 µg/ml), with exceptions of 1 LEV and 2 TET-resistant organisms, for which GEP MICs were unaffected. GEP was less active against 25 *Ureaplasma* spp. (MIC range 1–8 µg/ml), MIC_{90}=8 µg/ml. There was no effect on GEP MICs in 9 *Ureaplasma* spp. with resistance to LEV, AZI, and/or TET. GEP minimum bactericidal concentrations for 4 isolates of Mg, 4 Mh, 4 Mp, 3 Uu, and 1 Up were >3 dilutions > MICs, indicating bacteriostatic effect.

Conclusion GEP warrants further study to treat infections due to *Mycoplasma* spp., particularly organisms resistant to other antimicrobials as it was active against isolates resistant to AZI, TET, LEV, and/or MOX.