Town. One of the MSM reported a persistent urethral discharge which had failed to respond to previous therapy with oral cefixime. Agar dilution minimum inhibitory concentration assays were performed for eight antibiotics. The Johannesburg patients' isolates were further characterised by identification of key β-lactam-associated resistance mutations in *penA*, *mtrR* and its promoter, *porB1b*, *ponA*, and *pilQ* through PCR-based amplification and DNA sequencing. For molecular epidemiological characterisation, all three isolates were typed by *N. gonorrhoeae* multi-antigen sequencing typing (NG-MAST); additionally, full-length *porB* gene sequencing and multi-locus sequence typing (MLST) were performed for the Johannesburg isolates.

Results All three isolates were resistant to cefixime, ciprofloxacin, penicillin and tetracycline, intermediate/resistant to azithromycin but susceptible to ceftriaxone and gentamicin. The Johannesburg isolates had the type XXXIV *penA* mosaic allele in addition to previously described resistance mutations in the *mtrR* promoter (A deletion), *porB1b* (*penB*) (G101K, A102N) and *ponA1* (L421P). All three isolates had an identical *N. gonorrhoeae* multi-antigen sequence type (ST4822). The two Johannesburg isolates had an identical multi-locus sequence type (ST1901).

Conclusions All three strains were resistant to cefixime and were epidemiologically linked with identical NG-MAST sequence types. The Johannesburg isolates possessed a number of key β-lactam-associated resistance mutations and the type XXXIV *penA* mosaic allele. These two isolates belonged to a successful international MSM-linked multi-drug-resistant gonococcal clone (MLST ST1901), associated with several cefixime treatment failures in Europe and North America.

P1.021 A NATIONAL STUDY UTILISING THE SEQUENOM MASSARRAY IPLEX PLATFORM FOR HIGH THROUGHPUT MLST-BASED TYPING AND CHARACTERISATION OF RESISTANCE MECHANISMS IN NEISSERIA GONORRHOEAE

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Introduction Strain-typing and characterisation of associated resistance mechanisms is pivotal to understanding the development and spread of Neisseria gonorhoeae (NG) antimicrobial resistance (AMR). In Australia, we have embarked on a national study to determine the molecular basis of AMR in our local isolates with a view to implementing broad-based molecular surveillance for NG AMR.

Methods In this initial phase of the study, called GRAND (Gonorrhoea Resistance Assessment via Nucleic acid Detection), we are using the Sequenom MassARRAY iPLEX MALDI-TOF MS platform to characterise all available isolates (n = 2373) collected throughout Australia in the first half of 2012. To date, two iPLEX methods have been developed and validated: (1) a typing method targeting 14 informative SNPs previously shown to predict an MLST type; and (2) an AMR method targeting 11 common mutations associated with N. gonorrhoeae resistance to penicillin, ciprofloxacin, azithromycin and ceftriaxone, including important mutations on the penicillin binding protein (PBP2): A501 substitutions and the mosaic PBP2 sequence.

Results The results to date show that the technology is well suited for high-throughput typing of N. gonorhoeae isolates. In particular, we found it can be used on heat-denatured isolates (removing the need for a commercial DNA extraction kit) and can genotype (using both iPLEX reactions) up to 384 isolates within one working day for less than $AUS20.00 (€15.00) per isolate.

Conclusions The data from this study will provide pivotal information to inform the implementation of molecular-based NG AMR surveillance. Validation and testing is ongoing.

P1.022 HUMAN PAPILLOMAVIRUS 16 VARIANTS ANALYSIS IN MULTIPLE INFECTIONS

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Background/Objectives Human papillomavirus type 16 (HPV 16) is the primary aetiology of cervical cancer.

Risk factors associated to develop of malignant lesions include: infection persistence, specific HPV 16 variants and multiple infections presence.

We had characterised the genomic variability of E6, E7 and L1 genes in HPV 16 multiple infection patients samples and analysed the relationship between intratypic variants and lesion grade.

Methods HPV 16 multiple infection samples were amplified with three region type-specific primers and amplicons were sequenced using the "Big Dye Terminator Cycle Sequencing kit".

Sequences were aligned using Edit Sequence Alignment Editor and ClustalW, and compared with Genbank reported reference sequences: European (E), African (AF1 and AF2) and Asian-American (AA).

Lesions were divided as negative, low-grade (L-SIL) or high-grade (H-SIL).

Results HPV 16 multiple infections were identified in 125 samples and 78 of them were analysed for intratypic variations: 72 E variants (92.3%), 4 AA variants (5.1%), one AF1 (1.3%) and one AF2 variant (1.3%).

In E6 region, missense mutations (A104del and T350G) were defined in 59% and 41% of samples. In E7 region, a mainly synonymous variation (G849A, 41.33%) was detected. In L1 region, non-synonymous replacements were only identified: 6901insCAT (30%), 6902 insATC (65.7%) and GAT6951del (97.1%).

European variants were mainly detected in samples with no lesion while non-european variants were only found in H-SIL or L-SIL.

Conclusions E6, E7 and L1 genes are useful to determinate among E, AA and AF1/AF2 variants. Non-european variants are also present in our population.

Nucleotide variations different to define variants must be studied owing to their potential impact on pathogenesis. T350G nucleotide substitution is associated with elevated risk of cervical carcinomas. These variations should be taken into consideration.

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P1.023 MOLECULAR TYPING OF *TREPONEMA PALLIDUM* FROM AN ONGOING SYPHILIS OUTBREAK IN DENMARK

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Background Since 1999, the number of persons diagnosed with syphilis has increased dramatically in Denmark. Molecular typing was used to investigate the epidemiology of *Treponema pallidum* aiming to understand the dynamics of the epidemic. In recent years the tp0548 gene sequence has been used to further differentiate the subtypes obtained using the CDC typing system (number of 60-base pair

repeats within the *arp* gene and restriction fragment length polymorphism analysis of *tpr* subfamily II genes) into sequence types.

Methods *T. pallidum* PCR positive ulcer samples from 219 patients diagnosed in Denmark between 2009 and 2013 were identified for the study. At present, 16 specimens have been analysed and included in the study. Molecular typing was performed by sequence analysis of a 400-base pair region of tp0548 and will be supplemented with the CDC typing system.

Clinical data were obtained from the Danish National Syphilis Reporting System where patients diagnosed with early syphilis in Denmark are registered.

Results Sequence analysis of tp0548 revealed three sequence types designated f, g, and c, among the 16 patients. Four patients had tp0548 sequence type f. These patients were all men, and comprised both heterosexual men and MSM. One of the patients was originally from Iran. Eleven patients, including one female, had tp0548 sequence type g. These patients all reported Denmark as country of origin and the majority were habitants of Copenhagen. One patient had tp0548 sequence type c. This patient was a heterosexual man originally from Pakistan.

Conclusion These preliminary results show that a minimum of three tp0548 sequence types are prevalent in Denmark. To further differentiate between strain types, the clinical samples will be characterised using the CDC typing system. This study only included patients with lesions which limit our results to patients with early syphilis.

P1.024 *TRICHOMONAS VAGINALIS* DETECTION AND CHARACTERIZATION FROM WOMEN ATTENDING AN ANTI-RETROVIRAL CLINIC IN PRETORIA, SOUTH AFRICA

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Background Trichomoniasis caused by *Trichomonas vaginalis* affects both men and women, although the clinical presentation differs. Infected women are more likely to have symptoms compared to infected men. Approximately 75% to 100% of men are asymptomatic in comparison to approximately 50% to 75% of women who are identified as asymptomatic. In South Africa, data on the prevalence and detection of *T. vaginalis* is well-documented; however, data on the molecular characterization of *T. vaginalis* is limited. The study aimed to detect and characterise *T. vaginalis* isolates from HIV positive women attending an anti-retroviral clinic.

Methods Self-collected vaginal swabs from 380 HIV positive women were included in the study. *Trichomonas vaginalis* was detected using wet mount microscopy, culture and a commercial PCR kit. The genetic relatedness of 92 culture positive *T. vaginalis* isolates was determined. Five primers (TV1, TV2, TV3, TV5 and TV6) were used for the RAPD assay. The PCR-IGS-RFLP products were digested with five enzymes, namely: *Alu*I, *Hinf*I, *Rsa*I, *Sau*3AI and *Tsp*509.

Results A total of 8% (30/380) of specimens were positive for *T. vaginalis* using microscopy, 24% (92/380) of specimens were positive using culture and 31% (118/380) of the specimens were positive using the commercial PCR kit. RAPD assay analysis showed a high level of genetic diversity between the *T. vaginalis* isolates. The dendrogrammes obtained from the RAPD data grouped the 92 *T.vaginalis* isolates into between nine to 24 clusters with 70% similarity used to define clusters, while the PCR-IGS RFLP assay results for the isolates were genetically indistinguishable.

Conclusion The PCR assay was the most sensitive diagnostic tool for the detection of *T. vaginalis*. A high prevalence of *T. vaginalis*, consisting of different strain types, was detected in the HIV positive women included in the study.

P1.025 EFFECTS OF ABNORMAL VAGINAL FLORA AND MENOPAUSE ON CERVICOVAGINAL FLUID VISCOSITY

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Background Sexually transmitted pathogens, including HIV, are increased among women having abnormal vaginal flora. The mucus gel layer is a component of innate mucosal immunity, and the presence of mucus may contribute to the viscosity of genital tract fluid. Little is known about the impact of reproductive hormones, menopause and vaginal flora patterns on the viscosity of cervicovaginal fluid.

Methods Vaginal swabs and cervicovaginal lavage (CVL) were collected from 134 healthy asymptomatic post-menopausal women (n = 23), women in the follicular (n = 26) or proliferative (n = 19) phase, and women using levonogerestrol IUDs (n = 28), DMPA (n = 13) or combined oral contraceptives (n = 25). Vaginal smears were evaluated using the Nugent criteria. The viscosity (centipoise, cP) of each sample was measured in triplicate using a Cambridge MicroSample Viscometer. Student's t-test, analysis of variance, and linear regression were used to assess statistical significance.

Results The mean CVL viscosity among the 84 women having a Nugent score < 4 was 1.51(± 0.48) vs. 1.26(± 0.29) cP for the 50 women with abnormal vaginal flora (p = 0.001). There was no difference in CVL viscosity for women with intermediate vs. BV flora (p > 0.99). Similarly, there was no difference in viscosity among women at different phases of their menstrual cycle, nor among women using different hormonal contraceptive methods. However, the CVL of 23 postmenopausal women was less viscous compared to the 111 premenopausal women, 1.16(± 0.26) vs. 1.47(± 0.44) cP respectively (p = 0.001). In a linear regression model, abnormal flora (p = 0.01) and postmenopausal status (p = 0.005) were independently associated with decreased CVL viscosity.

Conclusion Abnormal flora and being post-menopausal are independently associated with decreased CVL viscosity. Even though phase of menstrual cycle and hormonal contraceptive use has been posited to have an impact on cervical mucus, these data suggest that these factors do not have a measurable impact of vaginal fluid viscosity.

P1.026 VALIDITY OF URINE-BASED DIAGNOSIS OF BACTERIAL VAGINOSIS

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Background Current gold standard diagnosis of bacterial vaginosis (BV) relies on categorising Gram-stained vaginal smears through Nugent scoring. We have recently described an alternative method of diagnosis, based on visualisation through fluorescence-in-situ-hybridisation (FISH) of the BV biofilm on desquamated vaginal epithelial cells present in urine sediments.

Methods A vaginal swab and a first void urine specimen were obtained from 72 pregnant women attending the antenatal clinic. The vaginal swab was used to assess the vaginal microbiota status through Nugent scoring. The urine specimen served for FISH-based diagnosis of the Gardnerella dominated polymicrobial adherent biofilms attached to desquamated vaginal epithelial cells.

Results Among the 12 women with BV on urine assessment, 10 had BV according to Nugent's score and 2 had intermediate microbiota. Presence of Gardnerella in a planktonic mode of growth occurred with 8 women and all have Nugent scores ≤3. Among the 52 women in which no Gardnerella could be documented through FISH, 2 had a Nugent score of ≥ 7, 2 a Nugent score of 4 to 6, and the remainder a Nugent score of ≤ 3. Accordingly, when comparing