

P2.030 UTILITY OF NEISSERIA GONORRHOEA POSITIVE APTIMA SPECIMENS TO ASSESS STRAIN DIVERSITY AND ANTIBIOTIC RESISTANCE

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Background Samples collected from community patients and patients visiting the Sexually Transmitted Infection (STI) clinics, submitted for the detection of *Neisseria gonorrhoea* (NG) in Alberta, Canada, are tested by the FDA approved APTIMA Combo 2[®] assay (GEN-PROBE). In addition swabs are collected from a limited number of high risk patients for culture and susceptibility testing. The isolates can also be used for molecular characterization of resistance determinants and strain typing. In order to gain a better understanding of the circulating sequence types (STs) and resistance determinants in cases where specimens are not collected for culture, such as community patients, a more practical approach would be to test the original sample submitted for Aptima testing by molecular methods.

Methods Sample pairs (specimen submitted for Aptima testing and culture within one day) from 26 patients including 22 males and 4 females ranging in age from 20 to 59 years were extracted using the QIAamp DNA mini kit and compared for direct molecular characterization. Samples were analysed for antimicrobial susceptibility based on mosaic changes that have been correlated with reduced susceptibility to extended-spectrum cephalosporins (ESC^{rs}) in the PBP2 protein coded by *penA*. Outer membrane proteins PIA and PIB coded by *porA/B* and *thpB* genes were sequenced and multi-antigen sequence typing (NG-MAST) was performed.

Results 100% sequence identity was observed between the sequences obtained from the Aptima and culture samples suggesting that direct molecular characterization can be performed from Aptima specimens. Based on the sequence data a strong correlation was observed between ESC^{rs}, *thp* allele 110, ST1407 and amino acid changes (G545S, I312M, V316T) in the PBP2 protein.

Conclusions This strategy will provide comprehensive surveillance data on circulating NG strains in lower risk populations and may help in guiding empiric treatment. Molecular methods can be enhanced for high throughput characterization of community samples.

P2.031 GRAM STAIN MICROSCOPY AS SYNDROMIC DIAGNOSTIC TEST TO EXCLUDE UROGENITAL GONORRHOEA IN HIGH RISK WOMEN IS LESS SENSITIVE WHEN OFFERED TO SYMPTOMATIC PATIENTS ONLY

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Background Before January 2010 at the Amsterdam STI clinic, Gram stain microscopy as syndromic test for urogenital gonorrhoea to enable direct treatment was performed in all high risk visitors irrespective of signs and/or symptoms. Since January 2010 Gram stain microscopy is performed only in those with signs and/or symptoms. We examined the diagnostic value of Gram stain microscopy as a syndromic test for urogenital gonorrhoea in high risk visitors with respect to signs and/or symptoms, by comparing the algorithm followed in the 2 periods.

Methods From January 2008–January 2010; 10,952 patients were included and from January 2010–January 2012 26,983. Urethral and cervical Gram stain results were compared with the standard gonorrhoea culture and sensitivity and specificity were calculated by sex. We

estimated the rate of lost to follow up cases (visitors with a false negative Gram result who did not come back for treatment) in the 2 periods. **Results** Gram strain microscopy had a high specificity, in both time periods regardless of sex (> = 99.8). Sensitivity decreased overall comparing years before and after 2010, from 87.2 to 84.8. This was mainly due to a sharp reduction of the already low sensitivity in women after 2010 from 32.0 to 23.1. Sensitivity in men remained high at 95.9 before 2010 and 95.4 thereafter. Loss to follow-up was estimated at 4.3% (2/47) before 2010 and 5.9% (8/135) after 2010. Overall, over 99% of all culture positive patients were treated.

Discussion The low sensitivity of Gram stain microscopy in females is known, but our results show that sensitivity to diagnose gonorrhoea is even lower when offered solely to symptomatic women. Although it did not have an impact on the number of treated infections, this counter-intuitive result could potentially lead to under-treatment and to be considered syndromic treatment policies.

P2.032 A NATIONAL QUALITY ASSURANCE SURVEY: LOW NEISSERIA GONORRHOEAE MISIDENTIFICATION RATES IN AUSTRALIA, 2012

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Introduction Commercial biochemical tests are typically used for the identification of *N. gonorrhoeae* isolates, however certain methods can exhibit specificity problems when testing commensal *Neisseria* strains. The aim of this study was to assess rates of phenotypic misidentification of *N. gonorrhoeae* in Australia.

Methods A total of 2373 isolates were received from reference laboratories throughout Australia in the first half of 2012, and comprised 98% of all gonococci isolated in Australia during this period. To confirm organism identity, all isolates were tested using in-house *N. gonorrhoeae* real-time PCR assays targeting multicopy *opa*, *porA* pseudogene and *cppB* genes.

Results The results showed that 98.5% (2336/2373) of isolates were positive for all three gene targets, 31 positive for two targets (3 *porA*-negative, 1 *opa* negative and 27 *cppB*-negative) and 6 were negative by all 3 PCR targets. Using 16S rDNA sequencing, 4 of the latter 6 isolates were determined to be non-gonococcal species (*Moraxella* and commensal *Neisseria*) whereas two isolates could not be identified. Using PCR as the reference standard, the results showed that phenotypic identification of *N. gonorrhoeae* in Australia is highly accurate at 99.8%.

Conclusions To date, this national quality assurance survey represents the most comprehensive assessment of phenotypic-based misidentification rates of *N. gonorrhoeae* isolates in Australia and elsewhere. Moreover, we demonstrated that Australian reference laboratories have maintained very low rates of phenotypic based misidentification (~0.2%). Further, the results highlight the need to use 2 distinct sequence targets for PCR-based detection of *N. gonorrhoeae* so as to avoid false-negative results.

P2.033 ISOLATION OF NEISSERIA GONORRHOEAE FROM THE TONSILS AND POSTERIOR OROPHARYNX USING CULTURE

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