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 *tlpB* genes were sequenced and multiantigen 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}, *tlp* 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 GONORRHOEA 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 GONORRHOEA FROM THE TONSILS AND POSTERIOR OROPHARYNX USING CULTURE

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Background Culture is insensitive for detecting pharyngeal gonorrhoea but is critical for determining antimicrobial resistance. Sampling technique appears to be important for optimal isolation of pharyngeal gonorrhoea, however, there are no published studies on the specific anatomical areas within the pharynx that should be targeted. The aim of this study was to compare isolation rates of gonorrhoea from the tonsils and posterior oropharynx.

Method Men who had sex with men attending the Melbourne Sexual Health Centre who screened positive for pharyngeal gonorrhoea using modified Thayer Martin medium were recalled and reswabbed prior to treatment. The repeat swabs consisted of careful swabbing from both tonsils followed by swabbing of the posterior oropharynx using a separate swab. These were plated onto separate media and cultured.

Results To date 61 MSM who screened positive for pharyngeal gonorrhoea have been recalled for repeat swabbing. The median interval between the initial positive screening test and repeat swabbing was 7 days. The positivity rates from the repeat swabs of the tonsils and posterior oropharynx were 69% (n = 41) and 52% (n = 32) respectively. The repeat tonsillar and posterior oropharyngeal swabs were both positive in 51% (n = 31) of men and were both negative in 30% (n = 18) of men. Eleven men (18%) had positive tonsillar and negative oropharyngeal results while one man had positive oropharyngeal and negative tonsillar results (p < 0.01). The results of concurrent nucleic acid amplification testing will be presented.

Conclusion These interim study results indicate that *Neisseria gonorrhoeae* can be cultured from the tonsils as well as the posterior oropharynx. While the positivity rate from tonsillar swabbing was higher than swabbing of the posterior oropharynx, sampling from only one of these sites had poor sensitivity. Many cases of pharyngeal gonorrhoea appear to be transient and self limiting.

P2.034 SAMPLING TECHNIQUE IS IMPORTANT FOR OPTIMAL ISOLATION OF PHARYNGEAL GONORRHOEA

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Background Culture is insensitive for the detection of pharyngeal gonorrhoea but isolation is pivotal to antimicrobial resistance surveillance. The aim of this study was to ascertain whether recommendations provided to clinicians on pharyngeal swabbing technique could improve gonorrhoea detection rates and to determine which aspects of swabbing technique are important for optimal isolation.

Methods This study was undertaken at the Melbourne Sexual Health Centre, Australia. Detection rates for pharyngeal gonorrhoea among clinicians were compared before (June 2006–May 2009) and

after (June 2009 - June 2012) recommendations on swabbing technique were provided. Associations between detection rates and reported swabbing technique obtained via a clinician questionnaire were examined.

Results The overall yield from testing before and after provision of the recommendations among 28 clinicians was 1.6% (134/8586) and 1.8% (264/15,046) respectively (p = 0.17). Significantly higher detection rates were seen following the recommendations among clinicians who reported: a change in their swabbing technique in response to the recommendations (2.1% versus 1.5%; p = 0.004); swabbing a larger surface area (2.0% versus 1.5%; p = 0.02); applying more swab pressure (2.5% versus 1.5%; p < 0.001); and a change in the anatomical sites they swabbed (2.2% versus 1.5%; p = 0.002). The predominant change in sites swabbed was an increase in swabbing of the oropharynx - from a median of 0% to 80% of the time.

Conclusion More thorough swabbing improves isolation of pharyngeal gonorrhoea using culture. Clinicians should receive training to ensure swabbing is performed with sufficient pressure and that it covers an adequate area that includes the oropharynx.

P2.035 AS NUCLEIC ACID AMPLIFICATION TESTING FOR *NEISSERIA GONORRHOEA* INCREASES IS CULTURE CONFIRMATION DECREASING?

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Background With the advent of nucleic acid amplification tests (NAATs) to detect *Neisseria gonorrhoeae* (GC) the use of selective culture is now reserved largely for symptomatic patients or confirmation of GC NAAT-positive results prior to treatment. The importance of culture remains greater than ever for monitoring antimicrobial susceptibility in the face of emerging GC resistance.

Aim We hypothesise that there is decreased likelihood of culture sampling at initial testing prior to treatment and thus there might be fewer culture samples available for antimicrobial susceptibility.

Methods A list of GC-positive cases in 2011 and 2012 was generated from the laboratory. All cases were reviewed and recorded as having had: culture not taken, culture taken & negative, culture taken & positive.

Annual cases were compared for the proportion being cultured prior to treatment, culture positivity rate and changes over time were assessed.

Results

Conclusion In our busy urban Genito Urinary Medicine (GUM) clinic we have shown that despite the increased ease and sensitivity of GC NAATs, GC culture has been sustained. The increase in the number of GC cases seen in 2012 is commensurate with an increase in testing episodes, yet the proportion with cultures taken pre-treatment actually increased from 64% in 2011 to 78% in 2012. Nonetheless, culture positivity has remained stable at 50% between 2011 and 2012. This is likely a result of our large MSM cohort, with high rates of rectal & throat infections in

Abstract P2.035 Table 1 Numbers of GC NAAT-diagnosed infections and associated culture results

	2011			2012		
	GC NAAT positive	GC culture taken	GC cultures positive	GC NAAT positive	GC culture taken	GC cultures positive
Total	1056	675 (64%)	336 (50%)	1325	1030 (78%)	518 (50%)
Rectum	408	235 (58%)	116 (49%)	509	386 (76%)	221 (57%)
Throat	424	239 (56%)	35 (15%)	556	400 (72%)	70 (18%)
Urine/urethral	222	201 (91%)	185 (92%)	260	244 (94%)	227 (93%)