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MOLECULAR SCREENING & AMP: QUANTIFICATION OF MYCOPLASMA GENITALIUM IN INFERTILITY PATIENTS

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Background *N. gonorrhoeae* and *C. trachomatis* are the predominant agents causing infertility. However, role of *M. genitalium* and *U. urealyticum* remain to be addressed. In addition, the association of load of these organisms with infertility is still not clear. The aim of the study was to screening and quantification of *M. genitalium*, *Ureaplasma* sp. *N. gonorrhoeae* and *C. trachomatis* in an infertile patients.

Methods A total of 248 women (98 infertile patients and 150 healthy control) who attended the infertility clinic and antenatal clinic of gynaecology department, were recruited in the study. Endocervical swabs (ECS) were collected from both group based on inclusion and exclusion criteria. For analytical sensitivity of uniplex real-time PCR (qPCR), targeted regions of reference strains were cloned in pGEMT Easy vector and transformed to JM109 *E. coli* cells. Cloned plasmid DNA were 10-fold diluted to determine the limit of detection for each organism and all clinical samples were tested and quantified.

Results Of 98 infertile patients, *M. genitalium* and *U. parvum*, *C. trachomatis*, *N. gonorrhoeae*, were detected in 7 (7.1%) and 42 (32.8%), 15 (15.3%), 8 (8.1%), respectively. Of 98 patients, 43.8% (43/98) had single infection and 19.3% (19/98) had mixed infection. *U. parvum* was the only detected organism in healthy control (30.7%). Our findings also suggest bacterial load of two classical agents (*C. trachomatis* and *N. gonorrhoeae*) and *M. genitalium* was not significantly associated with infertile patients. However, we observed *U. parvum* load was high in healthy control than in infertile patients but not was not stastically significant.

Conclusion In addition to traditional agents which causes infertility (*C. trachomatis* and *N. gonorrhoeae*), *M. genitalium* is also important cause and should be looked for in infertility cases though organism load was not found to be significantly associated with infertility. More studies are needed particularly in developing countries to study such associations.

Disclosure No significant relationships.

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ESTIMATING POPULATION BURDEN OF PELVIC INFLAMMATORY DISEASE DUE TO MYCOPLASMA GENITALIUM IN ENGLAND: AN EVIDENCE SYNTHESIS

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Background Increasing evidence indicates that *Mycoplasma genitalium* (Mgen) is a sexually-transmitted infection that can lead to pelvic inflammatory disease (PID) and possibly infertility. Resistance to azithromycin, which has been the first-line treatment, has been widely reported. To develop optimal testing and treatment guidelines, it is necessary to understand the

natural history of Mgen and the burden of associated disease. Several observational studies have provided valuable data, but no study has synthesized the available evidence to estimate the population burden of Mgen-associated disease.

Methods The POPI study was a chlamydia screening trial recruiting sexually active female students aged ≤ 27 years in London, 2004–2006. Women provided vaginal samples at baseline and follow-up, and were assessed for one-year incidence of PID by genitourinary doctors using participant questionnaires and medical records. Mgen infections were identified retrospectively from stored samples, using NAATs. We used the published data on Mgen prevalence, persistence of infection over median 16 (range 12–21) months' follow-up, and one-year incidence of PID in women infected or not infected with Mgen at enrollment. We conducted a Bayesian evidence synthesis using a simple (Susceptible-Infected-Susceptible) mathematical model of infection, with uninformative priors on all parameters.

Results In the POPI trial, 6.26% (1.82, 15.11)% (posterior median; 95% credible interval) of Mgen infections led to PID. We estimate that there were 1.96 (0.16, 6.27) new Mgen-related PID cases per 1000 women per year, and a total of 6728 (537, 21547) cases per year in 16–27-year-old English women. 10.8% (0.9, 33.0)% of the current burden of PID is caused by Mgen infection.

Conclusion Our model synthesises different types of data to understand the burden of Mgen infection and PID. Further data will be included to increase the precision of estimates, which are currently subject to wide uncertainty. We recommend studies in men, with urethritis as the disease outcome, which could be analysed with a similar model.

Disclosure No significant relationships.

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PLATFORM-AGNOSTIC REAGENTS FOR DETECTION OF MYCOPLASMA GENITALIUM

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Background *Mycoplasma genitalium* (MG), an STI of renewed interest, is associated with urethritis in men and cervicitis in women. Epidemiologic studies of MG infection outcomes have been limited by diagnostic capabilities. Recent molecular technologies have been applied to detection of MG-specific nucleic acid sequences. Use of commercially available assays leads to comparability across studies if the performance characteristics of these assays are known. The Aptima MG assay is available in some settings but requires access to assay-specific instrumentation. BioGX (Birmingham AL, USA) offers a custom lyophilized reagent for MG detection that is platform-agnostic and can be used in many clinical diagnostic settings. We compared the performance of the BioGX reagents to the Aptima MG (AMG) assay using specimens collected in support of a study of infertility in Cameroon.

Methods Vaginal samples were collected using Dacron swabs and stored in M4 transport medium. 200 μ L of M4 was loaded into an AMG transport tube or a BD MAX SBT

transport tube. Nucleic acid extraction and real-time PCR was performed on the BD MAX instrument using the BioGX Mycoplasma-Ureaplasma reagent. In this analysis we evaluated only the detection of MG.

Results Genital samples from 416 women were tested using both the AMG and BioGX reagents. The overall agreement was 99.3% ($\kappa=0.876$) with 402 negative and 11 positive samples in agreement. Two samples were positive only using the BioGX reagents and one specimen was only positive with AMG.

Conclusion In this MG detection study, we showed good performance of BioGX reagents on the BD MAX platform. The BioGX reagents can be used with any real-time PCR system, thus expanding diagnostic capacity to many laboratories. The performance in this study was very similar to that of AMG. Reagents capable of wider testing can facilitate epidemiologic studies designed to understand the impact of MG infection.

Disclosure No significant relationships.

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TESTING AND TREATMENT STRATEGIES FOR LIMITING DRUG RESISTANCE IN *MYCOPLASMA GENITALIUM*

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Background *Mycoplasma genitalium* (Mg) has rapidly increased its resistance to azithromycin, which has been first-line therapy for non-chlamydial-non-gonococcal urethritis (NCNGU), a proportion of which is due to Mg, and for treating Mg specifically. New commercial nucleic acid amplification tests (NAATs) are likely to greatly increase diagnosis and treatment of Mg, potentially promoting resistance. We previously developed the first transmission-dynamic model of Mg, which we now use to examine alternative approaches to NAAT testing and treatment.

Methods Our model synthesises evidence from surveillance data, and epidemiological and behavioural studies, and accounts for parameter uncertainty, including the fitness-costs and benefits of drug resistance. The model incorporates resistance due to de novo mutation and transmission. We examined scenarios regarding (i)targeting of NAAT (only testing symptomatic patients and partners vs testing all patients); (ii)using NAATs detecting resistance vs only detecting Mg; (iii)choice of first-line therapy (including continuing using azithromycin except where resistance has been detected vs alternative first-line regimen).

Results If azithromycin continues to be first-line therapy then resistance (and incidence of sequelae) will continue to rise, exacerbated by increased NAATs-based diagnoses. If asymptomatic screening occurs then resistance will increase 3.9(95% CI:2.1–5.7) times as rapidly as if only symptomatic patients and partners are tested. Pre-treatment resistance testing mitigates but does not prevent increases in resistance, due to resistance arising from frequent de novo mutation. Long-term outcomes of alternative regimens are highly uncertain.

Conclusion This work supports recommendations not to screen for Mg until a better treatment regimen has been determined. NAATs should include resistance-testing but this is not a

panacea. Improved understanding of Mg's natural history is urgently required, along with better surveillance of testing, diagnosis, and treatment, to monitor clinical adherence to guidelines, quantify drug-resistance fitness costs and benefits, and reduce uncertainty in decision-making. Internet-based testing and prescribing is a grave concern and needs to be controlled.

Disclosure No significant relationships.

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OH MG! THE SYMPTOMS OF *MYCOPLASMA GENITALIUM* IN WOMEN

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Background While the contribution of *Mycoplasma genitalium* (MG) to symptoms in men is well described, less is known about its clinical presentation in women. Data support an association with cervicitis, but an association with pelvic inflammatory disease is contentious. We undertook a study of 1200 symptomatic and asymptomatic women to determine the prevalence of MG and macrolide resistance, and to determine its association with common genital symptoms in women to inform indications for testing.

Methods Women attending Melbourne Sexual Health Centre from 18th April 2017 (in progress) were tested for MG and macrolide resistance (ResistancePlusMG SpeedX, Sydney), chlamydia and gonorrhoea (Aptima Combo 2, Hologic), trichomoniasis (microscopy and culture), bacterial vaginosis (BV) and candida (microscopy). Women underwent examination and completed a questionnaire on symptoms. The prevalence of MG, macrolide-resistance, STIs and coinfection, and association with genital symptoms and signs, was determined by univariate and multivariable analysis.

Results Of 1054 women enrolled to date (968 symptomatic and 86 asymptomatic), 62 women (6%, 95%CI 5–7%) tested positive for MG, with macrolide-resistance detected in 54% (95%CI 41–67%). Chlamydia and gonorrhoea were detected in 8% (95%CI 6–9%) and 1% (95%CI 1–2%) of the 1054 women respectively. Of the 62 women infected with MG, 42% (95%CI 30–55) also had BV, 26% (95%CI 16–38) candida, 6% (95%CI 2–16) chlamydia and 2% (95%CI 0–9) gonorrhoea. MG prevalence did not differ between symptomatic and asymptomatic women (6% vs 5%, $p=0.614$). No specific genital symptoms or signs were significantly associated with MG, in contrast to chlamydia, which was associated with post-coital bleeding (OR 1.7, $p=0.04$) and cervicitis (OR 2.3, $p=0.014$).

Conclusion MG was as common as chlamydia in our clinic population but in contrast to chlamydia was not associated with any specific clinical features that would inform testing practices. Macrolide resistance was detected in half of cases and coinfection with BV was particularly common.

Disclosure No significant relationships.