

Mycoplasma genitalium macrolide and fluoroquinolone resistance in pregnant women in Papua New Guinea

Mycoplasma genitalium is a sexually transmitted bacterium that colonises the human urogenital tract and in pregnant women has been associated with adverse pregnancy and birth outcomes.¹ In pregnancy, the prevalence of *M. genitalium* is estimated to be 0.9% (95% CI 0.6% to 1.4%) in high-income settings and 12.5% in individual studies in low- and middle-income-countries.^{1,2} Globally, *M. genitalium* antimicrobial resistance (AMR) continues to escalate,³ and despite rising rates of AMR the prevalence of macrolide and fluoroquinolone resistance in *M. genitalium* has not been widely studied among pregnant women.² Treatment of *M. genitalium* during pregnancy with selected antimicrobials such as macrolides is recommended, while tetracyclines are contraindicated and caution is recommended for fluoroquinolones,⁴ highlighting the potential issues surrounding available/effective antibiotics for this pathogen during pregnancy. In Papua New Guinea (PNG), macrolides and fluoroquinolones are routinely used for treatment of STIs⁴ and hence we aimed to characterise *M. genitalium* AMR in pregnant women.

We screened 69 *M. genitalium*-positive urine samples obtained from women attending their first antenatal clinic visit at five health facilities in Madang Province (PNG) between 2018 and 2019, enrolled in the control arm of a randomised controlled trial in PNG, the Women And Newborn Trial of Antenatal Interventions and Management.⁵ Confirmatory PCR was performed to verify the presence of *M. genitalium* in all 69 samples. Samples with positive results were then tested for the presence of AMR using targeted PCR assays. Macrolide and fluoroquinolone resistance/susceptibility markers were determined using previously published assays (online supplemental file). Stata V.15.1 was used to calculate the proportion of samples with resistance markers, with 95% exact binomial CI calculated using the Clopper-Pearson method. Where there were zero events, we give the 97.5% CI as the upper limit.

From the five study sites, all 69 samples that were initially reported to be positive for *M. genitalium* were confirmed as positive by repeat PCR testing. Of these, 58 (84.1%) samples were successfully characterised for macrolide resistance, while 54 (78.3%) were successfully characterised for fluoroquinolone susceptibility/resistance (table 1). In summary, no samples with available results harboured macrolide resistance mutations (0.0%, 97.5% CI 0.0% to 6.2%) or the S83I fluoroquinolone resistance mutation (0.0%, 97.5% CI 0.0% to 6.6%), and all 54 samples

were characterised as fluoroquinolone-susceptible (S83 wild-type).

While data on *M. genitalium* AMR remain limited in many regions, including in low-income countries of the Western Pacific region, our findings indicate no evidence of macrolide or fluoroquinolone resistance in PNG. This further supports the findings of two previous studies of *M. genitalium* in PNG (78 pregnant women) and Solomon Islands (56 non-pregnant women) that also reported no macrolide resistance.^{2,6} Some of the reasons behind the lack of resistance in *M. genitalium* in these regions may include the fact that current STI treatment guidelines in PNG do not stipulate testing and treatment of *M. genitalium* in symptomatic persons.⁴ Other studies have identified that incorrect screening practices leading to treatment with macrolides have contributed to *M. genitalium*'s increasing resistance to macrolides.⁷ Given that both macrolides and fluoroquinolones are prescription drugs in PNG, their use in treating STIs and other infections is highly regulated. These drugs are not available for use in self-medication and so the repeated consumption/use of these drugs is not likely to be driving the selection of resistance in PNG, as has been suggested in other regions of the world.

From an STI management perspective, the clinical symptoms of *M. genitalium* infection are not distinct nor fully understood, as *M. genitalium* can be found in conjunction with other STIs, including


Table 1 Results for macrolide and/or fluoroquinolone resistance in *Mycoplasma genitalium* isolates (N=69)

Study sites (location) (N=69)	Samples (n)	Average Ct value in <i>M. genitalium</i> PCR	SpeeDx ResistancePlus assay	TaqMan S83I and S83 assay		Summary
			Macrolide resistance	Fluoroquinolone resistance		
			23SRNA mutation	Wild-type*	S83I mutant	
Site 1 (rural), n=17	15	34.8	Not detected	Detected	Not detected	Characterised
	1	39.9	Not detected	No amplification	No amplification	Partially characterised
	1	38.5	No amplification	No amplification	No amplification	Not characterised
Site 2 (urban), n=11	6	35.5	Not detected	Detected	Not detected	Characterised
	1	37.9	Not detected	No amplification	No amplification	Partially characterised
	4	38.9	No amplification	No amplification	No amplification	Not characterised
Site 3 (rural), n=11	8	36.4	Not detected	Detected	Not detected	Characterised
	2	38.5	Not detected	No amplification	No amplification	Partially characterised
	1	38.5	No amplification	No amplification	No amplification	Not characterised
Site 4 (urban), n=11	8	36.8	Not detected	Detected	Not detected	Characterised
	1	39.2	Not detected	No amplification	No amplification	Partially characterised
	1	34.2	No amplification	Detected	Not detected	Partially characterised
	1	36.5	No amplification	No amplification	No amplification	Not characterised
Site 5 (rural), n=19	16	36.1	Not detected	Detected	Not detected	Characterised
	3	39.5	No amplification	No amplification	No amplification	Not characterised

Detected: sample was tested and we achieved an evaluable result (eg, no macrolide resistance observed and/or fluoroquinolone susceptibility was confirmed). No amplification: sample was tested; however, no evaluable result was obtained. Characterised: sample was successfully characterised by *both* assays. Not characterised: sample was *not evaluable* by *both* assays. Partially characterised: sample was successfully characterised by *one* assay.
*Wild-type (S83 sequence, no mutation) indicates fluoroquinolone susceptibility.

gonorrhoea and chlamydia. In PNG, symptomatic patients often present with more than one curable STI. In this setting, the targeted testing and treatment for specific STIs is not routine and instead syndromic management of STIs is the standard of care. Resource-constrained health facilities throughout PNG use a single-dose cocktail of antimicrobials, including augmentin, amoxicillin, probenecid, tinidazole and azithromycin/erythromycin. An increase in presumptive treatment of these infections could result in the overuse of macrolides, which could indirectly lead to the development of AMR in *M. genitalium*. Additionally, asymptomatic women in PNG are less likely to attend sexual health clinics and therefore unlikely to receive either macrolides or fluoroquinolones, reducing the potential for *de novo* AMR.⁸

It is important to report on findings where AMR has not been detected to reduce the risk of overestimation of *M. genitalium* resistance in these settings. Future studies of *M. genitalium* AMR in PNG and similar settings are warranted, given the potential for import of resistant infections or for *de novo* resistance, if the use of macrolides and fluoroquinolones for reproductive tract infections increases.

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Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by the Papua New Guinea Institute of Medical Research (IRB 1608) and the PNG Medical Research Advisory Committee (MRAC 16.24). Participants gave informed consent to participate in the study before taking part.

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1 **Supporting information**

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3 **Specimens**

4 Stored midstream urine samples, collected from women attending their first antenatal clinic visit at five
5 health facilities in Madang Province (PNG) between 2018 and 2019 were de-identified and stored at -80 °C
6 and transported to the University of Queensland Centre for Clinical Research (UQCCR, Brisbane,
7 Australia) for analysis. We identified 69 *M. genitalium* positive samples (unpublished data) from this cross-
8 sectional study and further analysed them as part of this study.

9 Urine samples were thawed at room temperature and nucleic acid extracted using the Qiagen DSP
10 virus/pathogen midi kit on the QIAasymphony SP/AS platform (Qiagen, Australia), according to
11 manufacturers' instructions with the Complex400-V3 DSP protocol.

12

13 **TaqMan Probe-based PCR assays**

- 14 i) MgPa screening: *M. genitalium* primers and probe from a previous publication were used to detect
15 *M. genitalium* within the clinical samples from this study.¹ In brief, reactions consisted of 12.5 µl
16 Quantitect Probe mastermix (Qiagen), 10pmol of each primer, 0.2 µM probe, 5 µL of nucleic acid
17 and PCR-grade water for a final volume of 25 µl. Samples were cycled using the Rotorgene 6000
18 (QIAGEN, Australia) real-time PCR instrument using: initial denaturation at 95°C for 15mins,
19 followed by 50 cycles of 95°C for 15 sec and 60 °C for 60 sec.
- 20 ii) Fluoroquinolone resistance assay: *M. genitalium*-positive samples were screened for the presence of
21 *parC* fluoroquinolone susceptibility and resistance markers using a previously developed assay.^{2,3}
22 Reactions included 10 µl SensiFast Probe master mix, 0.5 µM of forward and reverse primers, 0.2
23 µM of S83 wildtype (FAM-labelled) and S83I (G248T; HEX-labelled) probes and 3 µL of nucleic
24 acid extract in a total reaction volume of 20 µL. Each reaction included 5 µl nucleic acid extract and

25 15µl of the prepared TaqMan mastermix and analysed using the Applied Biosystems 7500 Fast Dx
26 Real-Time PCR instrument (Applied Biosystems®, Australia) using the following conditions: an
27 initial hold at 95 °C for 5 minutes, followed by 50 cycles of 95 °C for 15s and 60 °C for 60s,
28 acquiring data for both fluorescent probes on the annealing/ extension step.

29 **SpeedX ResistancePlus® MG PCR assay (Macrolide resistance)**

30 A commercial qualitative real-time PCR assay was used to identify *M. genitalium* and detect the five most
31 common mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, and A2059C,
32 *Escherichia coli* numbering) that are associated with resistance to azithromycin (a macrolide antibiotic).²
33 ⁴ According to the manufacturer's instructions, each reaction constituted of 10 µl of Plex mastermix (2x),
34 1 µL of 23S mix, 1 µL control mix, 5 µL of nucleic acid extract in a total reaction volume of 20 µL. The
35 reaction was analysed using the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument (Applied
36 Biosystems®, Australia) using the following conditions: initial denaturation of 95°C for 2min, followed
37 by a 10 cycle touch-down cycling (initial denaturation at 95 °C for 5 seconds (sec), then 61°C – 56.5°C (-
38 0.5 °C per cycle) for 30 sec) followed by 40 cycles of 95 °C for 5 sec and 52 °C for 40 sec (data
39 acquired). The selected channels for data acquisition included: FAM (*MgPa* gene), JOE (23S rRNA
40 mutation) and TAMRA (Extraction control).

41 Controls for PCR assays:

42 Well-characterised *M. genitalium*-positive clinical samples harbouring macrolide and fluoroquinolone
43 resistance markers served as controls in all PCR assays, and nuclease free water in place of nucleic acid
44 served as negative controls for all assays.

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