Title: Levels of *Mycoplasma genitalium* antimicrobial resistance differ by both region and gender in the state of Queensland, Australia: implications for treatment guidelines.

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Running title: *M. genitalium* antimicrobial resistance in Queensland

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Abstract:

Mycoplasma genitalium is frequently associated with urogenital and rectal infections, with the number of cases of macrolide resistant and quinolone resistant *M. genitalium* continuing to increase. In this study, we examined the levels of antibiotic resistance to these two common antibiotic treatments in geographically distinct locations in Queensland, Australia. Samples were screened for macrolide resistance-associated mutations using a commercially available kit (ResistancePlus™ MG; SpeeDx) and quinolone resistance-associated mutations were identified by PCR and DNA sequencing. Comparisons between antibiotic resistance mutations and location/gender were performed. Levels of *M. genitalium* macrolide resistance were high across both locations (62%). Quinolone resistance mutations were found in ~10% of all samples, with a number of samples harboring mutations conferring resistance to both macrolides and quinolones. Quinolone resistance was higher in Southeast Queensland, compared to North Queensland and this was consistent in both males and females (P = 0.007). Rectal samples from males harbored high levels of macrolide (75.9%) and quinolone (19%) resistance, with 15.5% harboring resistance to both classes of antibiotics. Overall the lowest observed level of resistance was for quinolones in females from North Queensland (1.6%). These data highlight the high levels of antibiotic resistance in *M. genitalium* within Queensland and the challenges faced by STI clinicians in managing these infections. The data do however show that levels of antibiotic resistance may differ between populations within the same state, which has implications for clinical management and treatment guidelines. These findings also support the need for ongoing antibiotic resistance surveillance and tailored treatment.

Introduction:

Mycoplasma genitalium was first identified in the urogenital tract of men in 1981 (1) and has since been confirmed as a sexually transmissible bacterium that is responsible for a range of sequelae. *M. genitalium* has been associated with acute and chronic urethritis in men, and can cause urethritis, cervicitis and pelvic inflammatory disease in women (2). Without appropriate treatment, *M. genitalium* infections are often chronic, with approximately 25% of infections persisting for >12 months, and some infections in women have been shown to persist for up to 2 years (3).

More recently, *M. genitalium* has gained widespread attention due to its increasing resistance to antibiotic treatments and as a consequence is now listed as a major emerging issue in the STI treatment guidelines published by the United States Centre for Disease Control and Prevention (4). Unlike other antibiotic resistant STI pathogens, such as *Neisseria gonorrhoeae*, for which antibiotic resistance increased somewhat steadily over time, the emergence and spread of antibiotic resistant *M. genitalium* appears to have occurred relatively quickly, and has prompted recent changes in the Australian and UK treatment guidelines. These guidelines now all recommend a 7-day course of doxycycline as empiric therapy for *M. genitalium* associated syndromes, and the use of a combined diagnostic-resistance assay where available to select an antibiotic to which the *M. genitalium* is likely to be susceptible (5). This resistance-guided approach involves the use of azithromycin for macrolide-susceptible infections or a quinolone, such as moxifloxacin or sitafloxacin, for macrolide-resistant infections to optimize cure (6, 7). However, there are still worrying trends in the levels of macrolide and quinolone resistant strains of *M. genitalium* that continue to threaten the efficacy of these treatment approaches.

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Macrolide resistance was first reported in *M. genitalium* in 2008 (8), and since this time the efficacy of these antibiotic treatments have diminished, with some studies reporting treatment success as low as 40% (4). The efficacy of moxifloxacin has also begun to decline, with recent meta-analyses showing a decrease in cure rates from 100% in studies conducted prior to 2010, to 89% in studies published after 2010 (9). The resistance mechanisms that underpin *M. genitalium* resistance to macrolides are well documented, enabling molecular methods to be developed to readily characterize the nucleotide mutations directly within *M. genitalium*-positive clinical samples. For macrolide resistance, five single nucleotide mutations at positions 2058 and 2059 within the macrolide resistance-determining region (MRDR) of the 23S rRNA gene are each independently associated with the failure of azithromycin (8). While resistance to quinolones has been associated with various mutations in the quinolone resistance-determining regions (QRDR) of the topoisomerase gene, *parC*, the contribution of specific *parC* mutations to treatment failure is still being determined. Similarly, the role of nucleotide mutations within the QRDR of the DNA gyrase gene, *gyrA*, is somewhat unclear with respect to clinical treatment failure (10-14).

In Australia, there have now been several studies examining *M. genitalium* antimicrobial resistance. In brief, these studies have shown that the current levels of macrolide resistance exceed 50% in the urban centers of Melbourne, Sydney and Brisbane (15-17) and 40% in backpacker populations in Northern Queensland (18), with sexual orientation being an important determinant of resistance, due to higher rates of antibiotic resistance in men who have sex with men (MSM) (19). Quinolone resistance, previously rare in *M. genitalium* in Australia, has been reported to be as high as 13.6% in a study from Melbourne in 2012 - 2013 (14, 20). In this current study, we further investigated and compared rates of both macrolide and quinolone antibiotic resistance among *M. genitalium* obtained from two distinct regions

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within Queensland: South-East Queensland (SEQ; which includes the cities of Brisbane [the capital of Queensland] and the Gold Coast) and North Queensland (NQ; including the city of Townsville, and surrounding regional and remote settings). Levels of resistance were further compared between males and females.

Methods:

Sample population:

This study was approved by the Children's Health Queensland human research ethics committee (HREC/12/QRCH/139). This retrospective study was performed on samples submitted to Pathology Queensland, Australia for the detection of *M. genitalium* by PCR between 2013 and 2017. The *M. genitalium* PCR utilized by Pathology Queensland was an in-house PCR method targeting the *M. genitalium* MgPa adhesin gene (15). Extracted DNA from *M. genitalium* positive samples was sent to The University of Queensland Centre for Clinical Research for screening for the presence of antibiotic resistance markers. The patients primarily comprised of those presenting to sexual health clinics with genital symptoms in whom testing for *M. genitalium* was indicated (e.g. urethritis, cervicitis, pelvic inflammatory disease and proctitis); Screening for *M. genitalium* is not currently recommended in the Australian guidelines. Samples used within this study including urine (n = 280), cervicovaginal swabs (n = 90), urethral swabs (n = 10), anal/rectal swabs (n = 60), throat swabs (n = 1) and samples from unknown sites (n = 6).

Antibiotic resistance screening:

Mutations (A2058G, A2058C, A2058T, A2059G and A2059C) within the macrolide resistance determining region (MRDR) of the 23S rRNA gene were detected using a

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commercially available kit (ResistancePlus™ MG Kit; SpeeDx; Sydney, Australia) (21-23). The kit is TGA-approved and CE-IVD marked, however is not currently for sale in the USA.

Mutations within the QRDR of the *gyrA* and *parC* genes were detected by PCR, using previously designed primers (14), and Sanger sequencing. Briefly, the Qiagen Quantitect SYBR kit (Qiagen, Australia) was used as the basis for the PCR reaction mix, and PCR products were submitted for DNA sequencing at the Australian Genome Research Facility (AGRF; Brisbane Australia). Alterations to the nucleotide sequences within the QRDR were compared to mutations that have been previously reported to be associated with clinical treatment failure or elevated minimum inhibitory concentration (MIC) values for quinolones in *M. genitalium* or other *Mycoplasma* spp. and *Ureaplasma* spp. (10-14).

***Statistics*:**

Comparisons across groups of categorical variables (e.g. gender, location) were performed using Pearson's Chi-square test. Statistical significance was accepted as $P < 0.05$.

Results:

Sample population:

A total of 524 *M. genitalium* positive samples were received from Pathology Queensland. Of these, 15 samples resulted in discordant results where we were unable to confirm the presence of *M. genitalium* and these were removed from our final dataset. We also identified 62 samples that were considered to be either a 'test of cure' (a patient sampled within 3 months of their initial presentation) or where a patient was sampled at multiple anatomical sites. These 62 samples were excluded from the primary analysis to determine the proportion of samples with macrolide and quinolone resistance, leaving 447 samples in the primary

141 analysis. Of these additional samples, 50 were included in an additional assessment
142 investigating the temporal changes in antibiotic resistance within individual patients (n = 50),
143 with the remaining 12 samples excluded from this secondary analysis as they were collected
144 from multiple anatomical sites of the same patient.
145
146 These 447 samples included 14 samples from 2013, 61 samples from 2016 and 374 samples
147 from 2017, representing 176 females and 269 males, with two individuals who did not
148 disclose their gender. Two hundred and nine samples originated from the SEQ region, while
149 238 *M. genitalium* samples originated from NQ. Of these, 26 samples did not have a
150 complete *gyrA* sequencing performed due to insufficient DNA however these remained
151 within our primary analysis as mutations in this region are currently of uncertain clinical
152 significance.
153
154 ***MRDR and QRDR mutations identified in samples:***
155 As the ResistancePlus™ MG assay was used to detect MRDR mutations and we did not
156 perform any DNA sequencing of the 23S rRNA gene, specific MRDR mutations were unable
157 to be reported. However, sequencing of the QRDR of *parC* and *gyrA* was undertaken and
158 specific quinolone resistance-associated amino acid changes were able to be reported. We
159 observed mutations within the topoisomerase gene, *parC*, and only two mutations of
160 uncertain clinical significance were observed within the DNA gyrase gene, *gyrA*. In
161 summarising the most common amino acid mutations observed within the study, we grouped
162 those mutations that were considered likely to be of clinical significance based on previous
163 publications that correlated quinolone mutations with treatment outcomes (20) and found that
164 the *parC* mutations S83I, D87Y and D87N were the most frequently observed mutations of
165 clinical significance and this was consistent across both SEQ and NQ (Table 1). The two

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gyrA mutations observed within this study were considered to be of uncertain clinical significance, as these were co-detected in *M. genitalium* which also harboured S83I *parC* mutations that are known to be associated with treatment failure (24).

Proportion of samples with MRDR and QRDR mutations:

Among the 447 patient samples, 277/447 (62.0%) carried strains which harboured MRDR, while a total of 47/447 (10.5%) samples harboured *M. genitalium* strains with *parC* or *gyrA* mutations in their QRDR. A total of 7.8% (35/447) patient samples harboured both MRDR and QRDR mutations (Table 2), herein referred to as dual class resistance. There was no evidence that the levels of MRDR or QRDR mutations changed over the study period (data not shown).

Regional differences in the levels of antibiotic resistance:

Both SEQ and the NQ regions of Queensland had similar proportions of samples with MRDR mutations (136/209; 65.1% and 141/238; 59.2%, respectively; $P = 0.21$). However, the levels of *parC*/*gyrA* mutations was significantly different between the two regions: SEQ *M. genitalium* samples were significantly more likely to harbour mutations associated with quinolone resistance (39/209; 18.7%), when compared to NQ (8/238; 3.4%) ($P < 0.001$; Table 2). Similarly, the proportion of *M. genitalium* samples harbouring mutations associated with dual class resistance differed, with SEQ having a significantly higher (28/209, 13.4%) rate of *M. genitalium* with dual class resistance, when compared to samples obtained from NQ (7/238, 3.0%; Table 2) ($P = 0.0001$).

Differences in the levels of antibiotic resistance according to gender:

Of the 176 samples from females within the study, 98 (55.7%) had MRDR mutations and 13 (7.4%) women had *parC* mutations. Of these women, only 8 (4.5%) harboured *M. genitalium* that had dual class resistance (Table 2). Macrolide resistance mutations were significantly higher in men, compared to women (P = 0.03), and this was consistent between both SEQ and NQ (Table 2). There was no significant difference in the frequency of QRDR mutations in men (34/269; 12.6%; Table 2), in comparison to women (13/176; 7.4%), and this was again consistent between both SEQ and NQ. Men and women from SEQ were more likely to harbour *parC* or *gyrA* mutations (men: 28/159 17.6%, women: 11/50, 22%) when compared to men and women in NQ (men: 6/110, 3.4%, women: 2/126, 1.6%; P = 0.007). Women from SEQ were also significantly more likely than women in NQ to harbour QRDR mutations (11/50, 22% vs. 2/126, 1.6%, respectively; P < 0.0001). Overall, the lowest observed level of resistance was for QRDR among females in NQ at 1.6% (Table 1).

Table 3 provides a summary of the sample types and associated levels of resistance. While sexual orientation data for men and women were not available for this study and we were unable to determine the true proportion of male samples that were from MSM; rectal samples from males likely represent MSM since this sample type are rarely, if ever, collected from heterosexual men. Urine samples within this study however likely represent a mixture of both MSM and heterosexual individuals. When comparing genitourinary samples from females to male rectal samples (a proxy for MSM), there were differences in the proportion of MRDR mutations from male rectal samples (44/58; 75.9%), compared to female genitourinary samples (93/167, 55.7%; P = 0.007) and we also observed significant differences in the levels of dual class resistance between male rectal samples (9/58, 15.5%) and female genitourinary samples (7/167, 4.2%; P = 0.03). Sample types with less than 3 specimens were not included in these comparisons.

Urine/urethral samples from males and urine/cervicovaginal samples from women harboured similar levels of macrolide (male: 177/269, 65.8%; female: 98/176, 55.7%, P = 0.1) and quinolone (male: 34/269; 12.6%; female: 13/176, 7.4%, P = 0.1) resistance; however, there was a significant difference in dual class resistance according to gender (male: 27/269. 10%; female: 8/176, 4.5%, P = 0.04). Specimen numbers for the remaining sample types were too low to make meaningful comparisons in relation to gender (Table 3).

Patients sampled over time had changes in their M. genitalium antibiotic resistance profile:

The results of the 50 patients sampled on multiple occasions; are summarized in Table 4. For 21/50 (42%) patients, the presence of antibiotic resistant *M. genitalium* appeared to persist following suspected treatment of the infection. The 'resistance profile' of male and female urine samples did not significantly differ from one another; however male rectal samples were more likely to have a 'persistent' antibiotic resistant profile (P = 0.017), in keeping with our previous finding that male rectal samples harbour high levels of antibiotic resistance. For 12/50 (24%) patients, the emergence of antibiotic resistant strains appeared following antibiotic treatment, which may be consistent with *de novo* antibiotic resistance, where baseline samples were susceptible to the antibiotic (Table 4, Post-treatment) but may also be associated with a new infection with *M. genitalium* with different susceptibility patterns. For example, patient 2 had no evidence of MRDR mutations prior to antibiotic treatment, while MRDR mutations were observed 22 days after treatment. Likewise, patient 10 had MRDR mutations at both pre- and post-treatment sample collection but no QRDR mutations; however, at post-treatment sample collection and S83I quinolone antibiotic resistance mutation was evident. In 28% (14/50) of patients, we observed a potential loss of antibiotic

240 resistance within the patient samples between the pre- and post-treatment sample collection
241 points; however, for two of these (patient 9 and 35) there was variation in resistance over
242 time: for both of these patients, there was a lack of MRDR mutations observed in sample 2
243 (post-treatment), however at the time a third sample was collected MRDR mutations were
244 again evident. It is important to note that due to the lack of clinical data this change in
245 resistance profile may also indicate reinfection of the patient with a susceptible strain of *M.*
246 *genitalium*. In 3/50 (6%), no change in the antibiotic resistance profiles was observed (Table
247 4).
248
249 **Discussion:**
250 Antibiotic resistance in *M. genitalium* has become a significant problem impacting upon the
251 successful treatment of these infections, and is epitomized by the fact that treatment failures
252 following recommended therapies are now commonplace (25). This is compounded due to
253 the paucity of available and effective therapies, and a lack of alternative antibiotic choices for
254 *M. genitalium*. Here, we present further evidence of the high rates of macrolide and
255 quinolone resistance and the emergence of dual class resistance in *M. genitalium* obtained
256 from Queensland, Australia, that show some regional and gender differences that likely
257 reflect differences in ethnicity and sexual orientation, which have significant implications for
258 clinical care.
259
260 Consistent with our previous pilot study conducted in Queensland [17], macrolide resistance
261 was high, with 62% of all samples harbouring mutations associated with macrolide
262 resistance. This level of macrolide-resistant *M. genitalium* is consistent with other estimates
263 in urban centers elsewhere, including Melbourne (16), Sydney (26), New Zealand (27), Japan
264 (24, 28, 29) and the United States (30) and these MRDR mutations have previously been

shown to be associated with elevated MIC values for macrolide antibiotics (8, 31) and clinical treatment failure with macrolides (32, 33). The level of quinolone resistance was approximately 10% across both SEQ and NQ, and is similar to recent reports from the Asia-Pacific region; for example, 10.7% of cases harbouring QRDR mutations in Japan (29) and 13.5% in Melbourne (20). Of note, we observed that 7.8% of *M. genitalium* cases harboured both MRDR and QRDR mutations consistent with resistance to both classes of antibiotics, which is also in-line with a recent study in Melbourne that reported dual class resistance was observed in 8.6% of specimens (20).

These data highlight the very real challenges faced by our STI clinicians in managing *M. genitalium* infections and helps explain why they continue to experience treatment failures. These data also reinforce the need for using combined diagnostic resistance assays to directly inform patient treatment, rather than relying on empiric treatment. In a recent study conducted in Melbourne, Read *et al.* were able to achieve cure rates of >92% (compared to macrolide treatment failure rates of 39% in a previous study (19)) where patients were tested for the presence of macrolide resistant *M. genitalium* at the time of presentation. The patients were treated with a 7-day course of doxycycline, followed by either azithromycin or pristinamycin, depending on the results of the mutation screening. The implementation of resistance testing-guided therapy has recently been shown to have considerable potential to improve first line cure of *M. genitalium* infections, and based on the results of our and other studies, a method that can rapidly detect QRDR mutations of known clinical significance may also be of considerable benefit to further enhance this approach.

Notwithstanding the above, our data also suggest that empiric treatment may still be viable in certain populations so long as adequate resistance surveillance data is available to directly

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inform local treatment practices and guidelines. While there was no significant difference in the rates of macrolide resistance between both SEQ (65.1%) and NQ (59.2%), there was much lower quinolone resistance levels in NQ (3.4%) compared to SEQ (18.7%). Notably, quinolone resistance was only 1.6% in females in NQ, suggesting that quinolones may be highly efficacious amongst heterosexuals in NQ. Further investigations are required to explore why quinolone resistance was significantly lower among females in NQ as compared with SEQ (22%), but it is likely the differences are attributable to differences in the populations, with the NQ region also including many Indigenous populations. Differences in antibiotic susceptibility for gonococci between Indigenous and non-Indigenous Australians are well-documented, with gonococci in many Indigenous communities remaining susceptible to antibiotics such as penicillin and ciprofloxacin that are no longer recommended for use elsewhere due to widespread resistance (34). Unfortunately, we did not have access to information on patient Indigenous status to allow such comparisons to be made for *M. genitalium*.

A further limitation of this study was the fact that we were unable to accurately correlate sexuality with our antibiotic resistance data. Studies elsewhere have shown an association between high rates of macrolide resistant *M. genitalium* and MSM (30, 35), with high rates of dual resistance to both macrolides and quinolones among MSM populations (30). It is, however, likely that many of the samples obtained from males within our study come from MSM, and this is supported by the fact that we observed high levels of macrolide resistance and dual class resistance in rectal samples from males (a sample type which represents MSM), when compared to genitourinary samples from females (more likely to represent heterosexual individuals) and this difference was statistically significant.

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Changes in resistance among patient samples were also observed over time, which in the absence of clinical data may represent individuals prior to antibiotic treatment and then following antibiotic treatment as a test of cure, but may also represent instances of reinfection with a new strain of *M. genitalium*. We observed that a 'persistent' antimicrobial resistant genotype was found more commonly in male rectal samples, than in samples from other anatomical sites, which also supports our previous finding that male rectal samples harbor high levels of antimicrobial resistance mutations. Future studies of interest would include pairing of molecular antimicrobial resistance typing alongside clinical data, as well as the use of molecular strain typing, in order to investigate the likelihood of reinfection and generation of resistance during antimicrobial treatments.

In summary, we have identified high levels of resistance to both macrolides and quinolones, as well as dual class resistance, among *M. genitalium* cases in Queensland. The data highlight the need for ongoing *M. genitalium* resistance surveillance as well as the importance of using molecular assays to tailor treatment of patients infected with *M. genitalium*.

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Contributions: E.L.S contributed to the design of the study; acquisition, analysis and interpretation of data, drafted the manuscript. E.T. assisted in the acquisition and analysis of

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data, C.B. assisted in the recruitment of patient samples, reviewed and provided feedback on the manuscript. C.S.B. provided critical feedback and clinical perspectives on the data within the paper, and assisted in drafting the manuscript. A.M. provided significant input into the clinical perspectives of *M. genitalium* infections, and provided feedback on the manuscript. F.F. assisted in sample collection and reviewed the final version of the manuscript. J.L-L. provided clinical insights into *M. genitalium* infections and reviewed/critically evaluated the manuscript. G.R.N. was instrumental in the provision of patient samples, critically reviewed and assisted in the drafting of the manuscript. D.M.W contributed to the design of the study, analysis and interpretation of the data and critically reviewed and assisted in the drafting of the manuscript. All authors approved the final submitted manuscript.

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457 **Figure and table legends:**

458 **Table 1.** Amino acid changes in *parC* and *gyrA* genes considered to be associated with
459 moxifloxacin failure. Amino acid position changes are reported according to the amino acid
460 positions within the *Mycoplasma genitalium* G37 genome.

461

462 **Table 2.** Levels of MRDR mutations and QRDR mutations associated with antibiotic
463 resistance by region and gender in Queensland, Australia. The 95% confidence interval is
464 listed in parentheses.

465

466 **Table 3.** Antibiotic resistance according to sample site and gender.

467

468 **Table 4.** Investigation of antibiotic resistance over time in 50 patients with multiple samples
469 submitted to Pathology Queensland.

Table 1. Amino acid changes in *parC* and *gyrA* genes considered to be associated with moxifloxacin failure. Amino acid position changes are reported according to the amino acid positions within the *Mycoplasma genitalium* G37 genome.

	***parC* amino acid changes of likely clinical significance[1]**	***parC* amino acid changes of uncertain clinical significance[2]**	***gyrA*[3] amino acid changes of uncertain clinical significance[2]**
South East Queensland			
Male (n = 26)	Ser → Ile 83 (n = 19), Asp → Asn 87 (n = 3), Asp → Tyr 87 (n = 3), Ser → Arg 83 (n = 1)	-	Gly → Cys 93 (n = 2)
Female (n = 11)	Ser → Ile 83 (n = 6), Asp → Asn 87 (n = 2), Asp → Tyr 87 (n = 1)	Ser → Asn 83 (n = 1), Asp → His 87 (n = 1)	-
Northern Queensland			
Male (n = 6)	Ser → Ile 83 (n = 4), Asp → Tyr 87 (n = 2)	-	-
Female (n = 2)	Ser → Ile 83 (n = 1), Ser → Arg 83 (n = 1)	-	-

[1] Clinical evidence from published data suggests that these mutations may be associated with treatment failure and/or elevated minimum inhibitory concentrations (MIC) to antibiotics.

[2] Uncertain clinical significance of these mutations with respect to treatment failure and/or elevated MIC data from published data.

[3] No known *gyrA* mutations were observed that were of known clinical significance.

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Table 2. Levels of MRDR mutations and QRDR mutations associated with antibiotic resistance by region and gender in Queensland, Australia.

The 95% confidence interval is listed in parentheses.

Region	Macrolide resistance mutations	Quinolone resistance mutations	Dual resistance mutations
South East Queensland (n = 209)	136, 65.1% (58 – 72%)	39, 18.7% (13.6 – 24.6%)	28, 13.4% (9.1 – 18.8%)
Male (n = 159)	109, 68.5% (61 – 76%)	28, 17.6% (12 – 24%)	22, 13.8% (8.9 – 20.2%)
Female (n = 50)	27, 54.0% (39 – 68%)	11, 22% (12 - 36%)	6, 12% (4.5 - 24.3%)
Northern Queensland (n = 238)	141, 59.2% (53 – 66%)	8, 3.4% (2 – 7%)	7, 3.0% (1.2 - 6.0%)
Male (n = 110)	68, 61.8% (52 – 71%)	6, 5.5% (2 – 12%)	5, 4.5% (1.5 - 10.3%)
Female (n = 126)	71, 56.3% (47 – 65%)	2, 1.6% (1 – 6%)	2, 1.6% (2 - 5.6%)
Undisclosed (n = 2)	2, 100.0% (16 – 100%)	0, 0.0% (0 – 84%)	0, 0.0% (0 - 84.2%)
TOTAL (n = 447)	277, 62.0% (57 – 67%)	47, 10.5% (7.8 – 13.7%)	35, 7.8% (5.5 - 10.7%)
Male (n = 269)	177, 65.8% (60 – 72%)	34, 12.6% (8 – 16%)	27, 10.0% (6.1 - 13.4%)
Female (n = 176)	98, 55.7% (48 – 63%)	13, 7.4% (4 – 12%)	8, 4.5% (2 - 8.8%)
Undisclosed (n = 2)	2, 100.0% (16 – 100%)	0, 0.0% (0 – 84%)	0, 0.0% (0 - 84.2%)

Table 3. Antibiotic resistance according to sample site and gender.

Sample Site and Gender	Macrolide resistance mutations	Quinolone resistance mutations	Dual resistance mutations
MALE URINE/URETHRAL SPECIMEN[1]	131/205 (63.9%)	23/205 (11.2%)	18/205 (8.8%)
FEMALE URINE/CERVICOVAGINAL SPECIMEN[1]	93/167 (55.7%)	12/167 (7.2%)	7/167 (4.2%)
ANAL/RECTAL SWAB			
Male (n = 58)	44/58 (75.9%)	11/58 (19.0%)	9/58 (15.5%)
Female (n = 2)	1/2 (50.0%)	1/2 (50.0%)	1/2 (50.0%)
TOTAL	***45/60 (75.0%)***	***12/60 (20.0%)***	***10/60 (16.7%)***
THROAT SWAB			
Male (n = 1)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)
TOTAL	***0/1 (0.0%)***	***0/1 (0.0%)***	***0/1 (0.0%)***
UNKNOWN SITE			
Male (n = 4)	2/4 (50.0%)	0/4 (0.0%)	0/4 (0.0%)
Female (n = 8)	4/8 (50.0%)	0/8 (0.0%)	0/8 (0.0%)
TOTAL	***6/12 (50.0%)***	***0/12 (0.0%)***	***0/12 (0.0%)***

[1] Two urine samples within this study were from patients who chose not to disclose their gender. These have not been included within the table, but both urine samples harboured macrolide resistance mutations, but no quinolone resistance mutations.

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Table 4. Investigation of antibiotic resistance over time in 50 patients with multiple samples submitted to Pathology Queensland.

Retested patients	Sample region	Specimen site (gender)	Sample number	No. of days between sample collections	Macrolide Resistance	Quinolone Resistance[1]	Dual Resistance	Resistance profile[2]
1	SEQ	Urine (M)	1	7	No	**Yes (D87N)**	No	Persistence
			2		No	**Yes (D87N)**	No	
2	SEQ	Urine (M)	1	22	No	No	No	Post-treatment
			2		**Yes**	No	No	
3	SEQ	Urine (M)	1	31	**Yes**	**Yes (S83I)**	**Yes**	Persistence
			2		**Yes**	**Yes (S83I)**	**Yes**	
4	SEQ	Urine (M)	1	127	**Yes**	**Yes (D87N)**	**Yes**	Loss
			2		**Yes**	No	No	
5	SEQ	Urine (M)	1	58	**Yes**	**Yes (S83I)**	**Yes**	Loss
			2		**Yes**	No	No	
6	SEQ	Urine (M)	1	35	**Yes**	No	No	Post-treatment
			2		**Yes**	**Yes (D87N)**	**Yes**	
7	SEQ	Urine (M)	1	28	**Yes**	No	No	Loss
			2		No	No	No	
8	SEQ	Rectal (M)	1	50	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
9	SEQ	Urine (M)	1	55	**Yes**	No	No	Loss
			2		No	No	No	
			3	16	**Yes**	No	No	
10	SEQ	Rectal (M)	1	176	**Yes**	No	No	Post-treatment
			2		**Yes**	**Yes (S83I)**	**Yes**	
11	SEQ	Rectal (M)	1	16	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
12	SEQ	Urine (M)	1	37	No	No	No	Post-treatment
			2		**Yes**	No	No	
13	SEQ	Urine (M)	1	18	**Yes**	No	No	Loss
			2		No	No	No	
14	SEQ	Rectal (M)	1	149	**Yes**	No	No	Loss

			2		No	No	No	
15	SEQ	Urine (M)	1	35	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
16	SEQ	Rectal (M)	1	29	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
17	SEQ	Urine (M)	1	48	**Yes**	No	No	Post-treatment dual resistance
			2		**Yes**	**Yes (S83I)**	**Yes**	
18	SEQ	Urine (M)	1	25	**Yes**	No	No	Loss
			2		No	No	No	
19	SEQ	Urine (M)	1	36	**Yes**	**Yes (S83I)**	**Yes**	Loss
			2		**Yes**	No	No	
20	SEQ	Urine (M)	1	29	No	No	No	Post-treatment
			2		**Yes**	No	No	
21	SEQ	Urine (M)	1	19	No	No	No	Post-treatment
			2		**Yes**	No	No	
22	SEQ	Urine (M)	1	25	No	No	No	No change
			2		No	No	No	
23	SEQ	Urine (M)	1	37	No	No	No	No change
			2		No	No	No	
24	SEQ	Urine (M)	1	113	**Yes**	**Yes (S83I)**	**Yes**	Loss
			2		**Yes**	No	No	
25	SEQ	Cervix (F)	1	31	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
26	SEQ	Urine (M)	1	169	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
27	SEQ	Rectal (M)	1	36	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
28	SEQ	Unspecified site (M)	1	43	**Yes**	No	No	Loss
			2		No	No	No	
29	SEQ	Vaginal (F)	1	40	**Yes**	No	No	Loss
			2		No	No	No	
30	NQ	Vaginal (F)	1	127	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
31	NQ	Urine (M)	1	80	**Yes**	No	No	Loss

			2		No	No	No	
32	NQ	Rectal (M)	1	98	No	No	No	Post-treatment
			2		**Yes**	No	No	
33	NQ	Urine (F)	1	124	No	No	No	No change
			2		No	No	No	
34	NQ	Urine (F)	1	34	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
			3	50	**Yes**	No	No	
35	NQ	Urine (M)	1	54	**Yes**	No	No	Loss
			2		No	No	No	
			3	43	**Yes**	No	No	
36	NQ	Vaginal (F)	1	103	No	No	No	Post-treatment
			2		**Yes**	No	No	
37	NQ	Rectal (M)	1	82	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
38	NQ	Urine (M)	1	113	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
39	NQ	Urine (M)	1	26	No	No	No	Post-treatment
			2		**Yes**	No	No	
40	NQ	Urine (unk[3])	1	37	**Yes**	No	No	Loss
			2		No	No	No	
41	NQ	Urine (M)	1	36	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
42	NQ	Urine (M)	1	9	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
43	NQ	Rectal (M)	1	28	**Yes**	**Yes (D87N)**	**Yes**	Persistence
			2		**Yes**	**Yes (D87N)**	**Yes**	
44	NQ	Urine (M)	1	41	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
45	NQ	Rectal (M)	1	45	No	No	No	Post-treatment
			2		**Yes**	No	No	
46	NQ	Urine (F)	1	42	No	No	No	Post-treatment
			2		**Yes**	No	No	
47	NQ	Rectal (M)	1	54	**Yes**	No	No	Persistence

			2		**Yes**	No	No	
			3	43	**Yes**	No	No	
48	NQ	Urine (unk[3])	1	39	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
49	NQ	Urine (M)	1	15	**Yes**	No	No	Persistence
			2		**Yes**	No	No	
50	NQ	Rectal (M)	1	29	**Yes**	No	No	Persistence
			2		**Yes**	No	No	

[1] Quinolone mutations within the *parC* topoisomerase gene, as per *M. genitalium* G37 amino acid numbering.

[2] Changes in resistance profiles from patient samples:

'Persistence' of resistance: indicates mutation persistently detected but in the absence of accompanying clinical data may reflect treatment failure, lack of treatment or reinfection

'Post-treatment' resistance: indicates the appearance of post-treatment (*de novo*) resistance mutations when the baseline sample was wildtype (no resistance mutation);

'Loss' of detectable resistance: indicates that baseline resistance and follow-up sample was wildtype. In the absence of clinical data this may reflect reinfection with susceptible strain or inability of assay to detect resistance mutation in a low load MRDR infection;

'No change' in resistance status: no changes observed in antibiotic resistance mutations;

[3] These patients chose not to disclose their gender.