Doxycycline and Sitafloxacin Combination Therapy for Treating Highly Resistant *Mycoplasma genitalium*

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*Antimicrobial-resistant Mycoplasma genitalium* is becoming increasingly common and creating major treatment challenges. We present early data on combination therapy with doxycycline and sitafloxacin to treat highly resistant *M. genitalium*. We found the regimen was well tolerated and cured 11/12 infections that had failed prior regimens with moxifloxacin and pristinamycin.

*Mycoplasma genitalium* is a sexually transmitted bacterium with marked capacity for developing antimicrobial resistance (1). Macrolides and 4th-generation fluoroquinolones, such as moxifloxacin, have been the main agents displaying efficacy against *M. genitalium*. However, macrolide resistance has increased to >50% in many nations, and quinolone resistance is increasing (2–6). In Australia, 16% of *M. genitalium* strains are reported to have dual-class resistance (5), and Japan reports dual-class resistance of up to 25% (2), resulting in infections that often cannot be cured with current recommended therapies.

Sequential monotherapy with doxycycline followed by moxifloxacin (7–9) is currently first-line therapy for macrolide-resistant *M. genitalium* in guidelines in Australia and the United Kingdom and achieves cure in 92% of cases (95% CI 88.1%–94.6%) at our service (7). When the doxycycline/moxifloxacin sequential regimen fails, we use a pristinamycin-based regimen, which achieves 75% cure (95% CI 66%–82%) (10). Since August 2017, for patients in whom both regimens failed, we administered a combination of 100 mg doxycycline and 100 mg sitafloxacin 2 times/day for 7 days.

Access to sitafloxacin is limited in many countries, but it is available in the Asia-Pacific region. Most publications on sitafloxacin are from Japan, where its use as a monotherapy is reported to cure ≈90% of *M. genitalium* infections (11). However, combination therapies can optimize cure and prevent further resistance in bacteria prone to developing resistance, such as *M. genitalium*. In vitro, a combination of doxycycline and sitafloxacin (doxycycline+sitafloxacin) shows synergy for quinolone-susceptible *M. genitalium* strains but has not been evaluated for highly resistant strains (J.S. Jensen, unpub. data). We provide early data on the efficacy and tolerability of a 7-day doxycycline+sitafloxacin combination therapy for treatment-resistant *M. genitalium*. The ethics committee of Alfred Hospital (Melbourne) approved this study (approval no. 232/16).

**The Study**

The study included 12 cases of macrolide-resistant *M. genitalium* detected among all patients assessed with the clinical protocol at Melbourne Sexual Health Centre (MSHC), Carlton, Victoria, Australia, for routine *M. genitalium* testing (7,9) during August 2017–April 2019 (Figure). During the study period, 96 (8%) of *M. genitalium* cases failed to respond to doxycycline/moxifloxacin; we subsequently treated 56 with pristinamycin, which failed in 15 (27%) patients. All 15 opted for combination therapy; 11 provided a test of cure, and the other 4 did not complete follow up. One...
additional patient also received combination therapy because her pelvic inflammatory disease (PID) did not respond to moxifloxacin. Our final analysis included 12 patients.

Among study participants, 9 sought treatment for urogenital symptoms and 1 for PID; 2 were asymptomatic contacts of persons with \textit{M. genitalium}. Median age was 29 years (interquartile range [IQR] 27–32 years). All men (10/12) had urethral infections; the 2 women had cervicovaginal infections.

We tested patient samples using the ResistancePlus MG Assay (SpeeDx, https://plexpcr.com). We defined treatment-resistant \textit{M. genitalium} as microbiological failure and persistent symptoms after first-line and second-line therapies failed. We classified microbial cure as \textit{M. genitalium} not detected and microbial failure as a positive result on test of cure 14–28 days after completing antimicrobial drug therapy.

At MSHC, we often give doxycycline before the main regimen to reduce \textit{M. genitalium} load and optimize cure (12). We term moxifloxacin-containing regimens as first-line, pristinamycin-containing regimens as second-line, and combination therapy as third-line (Table 1). All 12 patients received a moxifloxacin-based regimen; 9 had sequential doxycycline/moxifloxacin in keeping with clinical guidelines (9). The other 3 had moxifloxacin alone, 1 for PID, and 2 received treatments prior to coming to MSHC (1 was treated for 10 days and the other for 30 days, but we do not know the physician’s rationale for treatment duration). Moxifloxacin-based regimens failed in all patients; 11 were then treated with pristinamycin, which also failed. We administered combination

\textbf{Figure.} Clinical approach and treatment for patients with diagnosed macrolide-resistant \textit{Mycoplasma genitalium} at Melbourne Sexual Health Centre, Australia. PID, pelvic inflammatory disease; STI, sexually transmitted infection. \textsuperscript{*}Routine testing with the ResistancePlus MG assay (SpeeDx, https://plexpcr.com). \textsuperscript{†}Moxifloxacin 400 mg/day for 14 days. \textsuperscript{‡}Doxycycline 100 mg 2 times/day for 7 days, then moxifloxacin 400 mg/d for 7 days. \textsuperscript{§}Test of cure was recommended 14–28 days after completing antimicrobial treatment and all patients received a reminder. ResistancePlus MG Assay was used for all tests of cure. \textsuperscript{¶}When sequential therapy failed, patients were given a pristinamycin-based regimen for 10 days, either 1 g 4 times/day alone or 1 g 3 times/day in combination with doxycycline 100 mg 2 times/day. Doxycycline pretreatment also was given to some patients. \textsuperscript{♯}Doxycycline 100 mg and sitafloxacin 100 mg taken together 2 times/day for 7 days.
therapy without preceding pristinamycin to 1 patient because of concerns regarding her PID.

All patients received doxycycline+sitafloxacin combination therapy for 7 days; 9 had preceding doxycycline for varying durations (Table 1). Among 12 patients, 11 (91.7%; 95% CI 64.9%–98.5%) were cured and achieved complete symptom resolution after combination therapy. Combination therapy failed in 1 patient who experienced persistent dysuria. Median time to test of cure after combination therapy was 20 (IQR 14–24) days. Median duration from first M. genitalium diagnosis to cure was 125 (IQR 106–144) days. Before test of cure, all patients were classified as no- or low-risk for reinfection by the treating clinician on the basis of no sex or 100% condom use with any partner or sex with a fully treated partner in the interval between treatment and test of cure (7,12).

All patients whose first-line and second-line therapies failed were symptomatic, including the 2 who initially were asymptomatic contacts. All 10 men reported persistent fluctuating dysuria, 6 reported urethral discharge, 2 urethral irritation or itching, and 1 mental inflammation. Both women reported fluctuating abnormal vaginal discharge, 1 reported intermittent dysuria, and the patient with PID reported persistent dyspareunia. Men typically experienced a stepwise reduction in urethral symptoms after commencing antimicrobial drugs, but dysuria re-emerged during follow-up.

Among 10 patients for whom adherence and adverse effects were available, 9 (90.0%; 95% CI 60.0%–99.5%) reported taking all doses of both drugs, including the patient whose treatment failed; 1 reported missing 1 tablet of sitafloxacin. Six (60.0%; 95% CI 31.3%–83.2%) patients reported no adverse effects. Among the other 4, adverse effects were mild and resolved spontaneously (1 each of diarrhea, arthralgia, tendon pain, and possible blurred vision).

Sanger sequencing of the quinolone resistance-determining regions of the parC and gyrA genes revealed single nucleotide polymorphisms for parC in all cases and for gyrA in 5/12 cases before combination therapy (Table 2). The parC mutations corresponded to amino acid changes S83I (G248T; n = 11) and D87N (G259A; n = 1). The gyrA mutations corresponded to amino acid changes M95I (G285A; n = 3), A79S (G235T; n = 1), and D99N (G295A; n = 1). In 1 case, a gyrA mutation appeared to develop after moxifloxacin failure (case 9; Table 2). The patient in whom combination therapy failed had a single parC S83I change detected (Table 2).

Conclusions
Combination therapy with doxycycline+sitafloxacin was well tolerated and effective against treatment-
resistant *M. genitalium*. The regimen was acceptable to clinicians and is now used as our third-line regimen.

Moxifloxacin failure has been associated with specific polymorphisms in the quinolone resistance-determining regions of *parC* (2,5). The *parC* G248T mutation, which causes amino acid change S83I, is the most common mutation associated with moxifloxacin failure (5). The less common G259A(D87N) mutation has been associated with higher moxifloxacin MICs in 3 *M. genitalium* strains (J.S. Jensen, unpub. data). S83I contributes to both sitafloxacin and moxifloxacin failure (5). Although sitafloxacin is more likely than moxifloxacin to cure an infection carrying an S83I mutation, we previously found concurrent *gyrA* mutations, particularly M95I, increased the risk for sitafloxacin failure (5). In this study, moxifloxacin failed in all 12 cases with a *parC* mutation and 5 had a concurrent *gyrA* mutation. However, 11/12 cases were cured with the doxycycline+sitafloxacin combination. Of note, the 1 treatment failure occurred in a case with only the *parC* G248T/S83I mutation, no concurrent *gyrA* mutation, and no more detectable resistance than cured cases.

Of note, sitafloxacin alone might have cured some or all infections. Further studies comparing sequential and combination therapy with doxycycline+sitafloxacin in highly resistant *M. genitalium* are needed. However, treatment failures, particularly in cases with concurrent *parC* and *gyrA* mutations, would be expected. Moreover, the variable duration of preceding doxycycline may have improved cure.

In conclusion, our results provide important early data on the efficacy and tolerability of doxycycline+sitafloxacin combination therapy to cure highly resistant *M. genitalium* infections. This approach could become part of a broader stewardship strategy to evaluate combination therapy, which might be needed to further prevent development of antimicrobial-resistant *M. genitalium*.

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