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Combination Therapy for Multi-Drug-Resistant *Mycoplasma genitalium*

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Combination Therapy for Multi-Drug-Resistant *Mycoplasma genitalium*

Running title: Combination therapy MDR *Mycoplasma genitalium*

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Abstract: In Belgium, around a quarter of *M. genitalium* infections are resistant to both macrolides and fluoroquinolones -termed multi-drug-resistant (MDR) infections. It is unclear what the best treatment is for these infections. We report the first two cases of MDR *M. genitalium* urethritis treated with combination therapy of minocycline, metronidazole, methenamine and pristinamycin. In both cases, this treatment resulted in microbiological and clinical cure.

Keywords: *M. genitalium*; minocycline; metronidazole; methenamine; pristinamycin; AMR

Background

Mycoplasma genitalium is becoming increasingly resistant to first- and second-line treatments. In Belgium, the prevalence of macrolide resistance in *M. genitalium* varies between 100% in men who have sex is 100% and 48% in woman [1]. Around a quarter of infections are resistant to both macrolides and fluoroquinolones -termed multi-drug-resistant (MDR) infections [1]. The European IUSTI treatment guidelines suggests trying doxycycline or minocycline 100 mg BID for 14 days (oral) or pristinamycin 1 g QID for 10 days (oral) [2]. Others have suggested a variety of other treatments such as chloramphenicol, sitafloxacin, and metronidazole or sequential tetracycline followed by azithromycin or moxifloxacin . These treatments, however, fail frequently in MDR infections [2]. Monotherapy, even if sequential leads to the further selection of antimicrobial resistance (AMR). To prevent the emergence of this AMR and to improve treatment success, we have attempted combination therapy for MDR *M. genitalium* infections. We used a regimen of pristinamycin, minocycline, methenamine and metronidazole. Methenamine-amygdalate is a urinary antiseptic that has been successfully been used to prevent recurrent urinary tract infections [3]. It undergoes hydrolysis in the acidic urine where it is converted into formaldehyde, which exhibits antimicrobial activity by denaturing proteins and nucleic acids within bacterial cells [3]. A daily dose of 2 g of methenamine results in a urine concentration of 18–60 µg/mL of formaldehyde, which exceeds the MICs of urinary pathogens [4]. We gave the methenamine at a dose of 1g QID x 4 weeks. It has been shown to be safe for daily use for at least 12 months [3]. Methenamine has not been evaluated for activity against *M. genitalium*. It is also not active against intracellular bacteria [3] and since *M. genitalium* is known to reside intracellularly [5], we considered it unlikely that methenamine would be able to eradicate *M. genitalium*. We therefore added pristinamycin, minocycline and metronidazole to methenamine-amygdalate for the first 14 days of treatment.

Case 1

Our first case was a 27-year-old man who has sex with men and takes HIV PrEP intermittently. He presented with a urethral discharge in October 2021. Genital examination revealed a purulent

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urethral discharge. Nucleic acid amplification (NAAT) of a first-void urine specimen was negative for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* but positive for *M. genitalium*. He was treated with doxycycline 100mg twice daily for 7 days for non-gonococcal urethritis with only temporary improvement in his symptoms (Table 1). Over the subsequent two and a half years he was treated with multiple courses of doxycycline, minocycline, azithromycin, moxifloxacin, pristinamycin, chloramphenicol and metronidazole with at best temporary resolution of his symptoms (Table 1; Figure 1). In February 2024 he received triple therapy with minocycline, metronidazole and pristinamycin for 14 days with rapid return of his symptoms 1 day following treatment cessation. In March 2024, we commenced quadritherapy with methanamine, minocycline, metronidazole and pristinamycin as detailed in Table 1. His symptoms resolved within 7 days and have not returned. NAAT testing of his urine in July 2024 was negative for *M. genitalium*.

At the beginning of his infection, his main-partner was found to be negative for *M. genitalium*. Since June 2022 he has only had sex with a small number of other men. This sex was receptive oral sex without a condom.

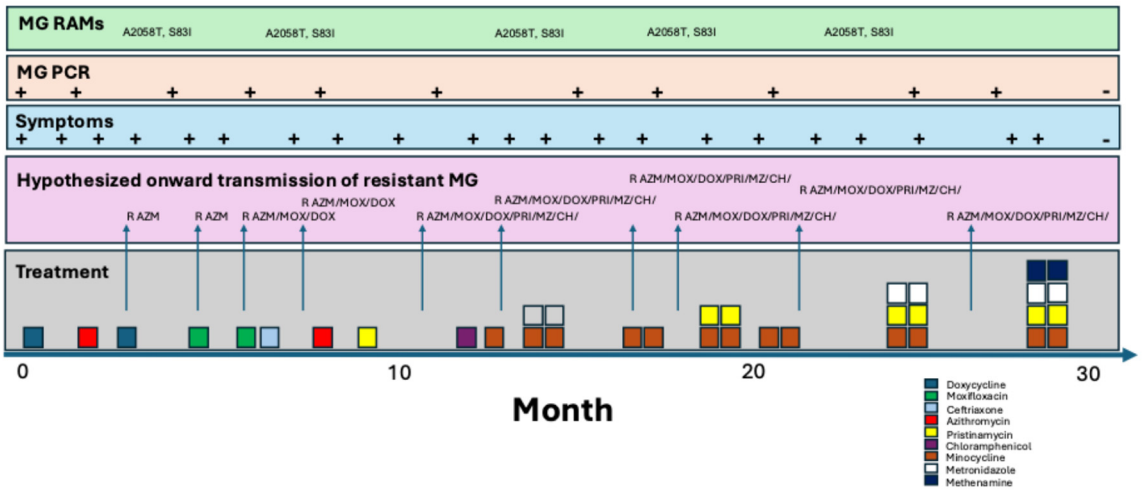

Figure 1. Time of symptoms, *M. genitalium* detection and treatments given to case 1.

Table 1. Summary of symptoms, *M. genitalium* molecular test results and antimicrobial treatments of case 1.

Date	Symptoms	Micro. (PMN/HPF)	PCR MG	RAMs	Treatment	Outcome
10/2021	Subtle DC, dysuria		+		Doxycycline 100mg BID x 7d	Initial improvement but symptoms return 2 days post treatment
02/2022	Subtle DC, dysuria	5-10	+		Azithromycin 500mg d1 and 250mg d2-5	No improvement
06/2022	DC, dysuria		+	A2058T, S83I	Doxycycline 100mg BID x 21d	No improvement
07/2022	DC, dysuria		+	A2058T, S83I	Moxifloxacin 500mg BID x 10d	Symptoms return 10days post treatment

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08/2022	DC, dysuria		+	A2058T, S83I	Moxifloxacin 500mg BID x 10d + ceftriaxone 1g IMI (partner had NG)	No improvement
09/2022	DC, dysuria		+		Azithromycin 500mg d1 and 250mg d2-5 +BPG 2.4mu (partner had syphilis)	No improvement
10/2022	DC, dysuria		+	A2058T, S83I	Pristinamycin 1g QID x 10d	Symptoms return 21 days post treatment
12/2022	DC, dysuria		+	A2058T, S83I	None	
02/2023	DC, dysuria		+		Ibuprofen for pain	
03/2023	DC, dysuria	8	+		Chloramphenicol 1g QID x 14d	
04/2023	DC, dysuria	4	+		Minocycline 100mg BID x 14d	Symptoms return 20 days post treatment
05/2023	DC, dysuria	10	+	A2058T, S83I	Minocycline 100mg BID x 14d then metronidazole 500mg TID x 14d	All symptoms resolve except light dysuria x 30 days
08/2023	DC, dysuria		+		Minocycline 100mg BID x 14d then Pristinamycin 1g QID x 10d	No response
11/2023	DC, dysuria		+		Minocycline 100mg BID x 14d	
01/2024	Dysuria		+		Minocycline 100mg BID x 14d	
02/2024	DC, dysuria		+		Minocycline 100mg BID x 14d + metronidazole 500mg TID x 14d + Pristinamycin 1g QID x 14d	Symptoms return 1 day post treatment

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03/2024	DC, dysuria		+		Minocycline 100mg BID x 14d + metronidazole 500mg TID x 14d + Pristinamycin 1g QID x 14d + methenamine-amygdalate 1g QID x 28d	Symptoms resolve within one week
07/2024	None	0	Neg			No symptoms
09/2024	None					No symptoms

DC – urethral discharge; BID -twice daily; TID -three times daily; QID -four times daily;.

Case 2

The second case was a 56-year-old man who has sex with men and women and takes HIV PrEP intermittently. He presented in January 2024 with dysuria and a purulent urethral discharge. Nucleic acid amplification of a urine specimen was positive for *M. genitalium* (xx) and negative for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (xx) (Table 2). Various courses of doxycycline, azithromycin, moxifloxacin were administered over the course of the subsequent 6 six months with only temporary symptomatic improvement. Molecular testing confirmed mutations known to cause resistance to macrolides and fluoroquinolones ().

Table 2. Summary of symptoms, *M. genitalium* molecular test results and antimicrobial treatments of case 2.

Date	Symptoms	Micro. (PMN/HPF)	PCR MG	RAMs	Treatment	Outcome
01/2024	DC, dysuria	15+	+		Doxycycline 100mg BID x 7d then azithromycin 500mg d1 and 250mg d2-5	No improvement
02/2024	DC, dysuria	5-10	+		Doxycycline 100mg BID x 21d, then then Moxifloxacin 500mg BID x 7d	Symptoms return 1 day post treatment
03/2024	DC, dysuria		+		Azithromycin 2.5g over 4d then Moxifloxacin 500mg BID x 7d	No improvement

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04/2024	DC, dysuria				Doxycycline 100mg BID x 7d then Moxifloxacin 500mg BID x 10d	Symptoms return 10 days post treatment
05/2024	DC, dysuria		+		Doxycycline 100mg BID x 14d then Azithromycin 500mg d1 and 250mg d2-5 then metronidazole 500mg TID x 7d	No improvement
06/2024	DC, dysuria		+		Doxycycline 100mg BID x 28d	No improvement
07/2024	DC, dysuria		+		Minocycline 100mg BID x 14d + metronidazole 500mg TID x 14d + Pristinamycin 1g QID x 14d + methenamine-amygdalate 1g QID x 28d	Symptoms resolve within 10days of starting treatment
09/2024	None	0	-		None	No symptoms

DC – urethral discharge; BID -twice daily; TID -three times daily; QID -four times daily;.

In July 2024 he commenced the same quadritherapy regimen as case one, with resolution of his symptoms within 10 days. A urine NAAT conducted 4 weeks post treatment cessation was negative for *M. genitalium*. His female main-partner was asymptomatic but her urine tested positive for *M. genitalium* in January 2024. Resistance testing was not performed. She was treated with sequential azithromycin (500mg day one then 250mg days 2 to 5) and then moxifloxacin 500mg BID x 7 days. A NAAT test performed 1 month after treatment cessation was negative for *M. genitalium.*

Discussion

In both cases, quadritherapy was associated with the rapid cessation of symptoms and microbiological cure. Both cases had confirmed *M. genitalium* infections that were resistant to macrolides and fluoroquinolones. Both individuals had tried multiple courses of antimicrobials without success. In the second case, the quadritherapy involved two new treatments – pristinamycin and methenamine – which could have been responsible for treatment success. The only treatment not used prior to quadritherapy in case one was methenamine. The inclusion of methenamine may thus have been important in treatment success for both cases.

Our findings are however based on two case reports and due caution is therefore required. It is possible that the clearance of *M. genitalium* was due to natural clearance of the infection, due to the sequential antimicrobial therapies or some other factor. Randomized controlled trials are urgently required to build an evidence base for the optimal treatment of MDR *M. genitalium*. In vitro

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evaluations of the antimicrobial susceptibility of these agents alone and in combination would also be useful. A key problem here is that methenamine is only active in an acidic milieu [3]. This acidic milieu is however toxic to most of the cell lines used to cultivate *M. genitalium* [6].

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