Initial failure of pristinamycin treatment in a case of multidrug-resistant Mycoplasma genitalium urethritis eventually treated by sequential therapy

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TITLE

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SHORT SUMMARY

The initial failure of pristinamycin-based therapy was possibly linked to the presence of A2062T mutation, and the *Mycoplasma genitalium* infection was finally cured after secondary loss of this mutation.

ABSTRACT

We present a case of persistent *Mycoplasma genitalium* (MG) urethritis with documented macrolide and fluoroquinolone resistance, and we describe the A2062T mutation in the 23S rRNA gene, possibly associated with pristinamycin resistance. After several treatment failures and loss of the A2062T mutation, MG urethritis was finally cured by a sequential antibiotic treatment including minocycline.

INTRODUCTION

Mycoplasma genitalium (MG) is the most common cause of urethritis after *Neisseria gonorrhoeae* (NG) or *Chlamydia trachomatis* (CT). European treatment guidelines recommend azithromycin 1.5 g (500 mg followed by 250 mg daily for a total of 5 days) , and moxifloxacin (400 mg daily for 7-10 days) as second-line treatment or in case of uncomplicated macrolide resistant MG infection.[1] However in less than 10 years, more than 50% of strains have become macrolide resistant and 5-10% fluoroquinolone resistant, in some parts of the world.[2,3]

Hence sequential antibiotic treatment following susceptibility testing was suggested for MG urethritis.[4,5] Treatment began with doxycycline for 7 days meanwhile possible macrolide resistance was detected. According to susceptibility testing, patients then received azithromycin for 5 days or fluoroquinolones (sitafloxacin or moxifloxacin) for 7 days in case of macrolide resistance. The rationale of sequential therapy beginning with doxycycline is to reduce the bacterial load, to optimize the second antibiotic's effect while limiting the emergence of resistance.

We report a case of multidrug-resistant MG urethritis successfully cured by several sequential antibiotic treatments guided by susceptibility testing.

CASE REPORT

The patient was a 51-year-old Parisian man having sex with men, who had just started HIV pre-exposure prophylaxis (PrEP). He consulted a general practitioner about urethral symptoms (dysuria and discharge). After performing NG and CT nucleic acid amplification tests (NAATs) on urine specimen, he received empirical therapy combining 7 days oral cefixime plus 1 g single dose azithromycin (Table). NG and CT NAATs were negative. His symptoms persisted 12 days later.

He was referred to a STI clinic, where the MG NAAT performed was positive. Moxifloxacin was given but stopped after 48 hours because the patient reported tendon pain in lower limbs, as a possible side effect of fluoroquinolones. He was given a sequential treatment with doxycycline for 7 days followed by pristinamycin for 14 days, during which symptoms decreased but relapsed afterwards.

Since his urethral discharge persisted, he was referred to our Infectious Diseases department, at Pitié-Salpêtrière hospital in Paris. MG NAAT remained positive. Amplification and Sanger sequencing of domain V of the 23S rRNA gene[6] identified two mutations. The first one was A2058T substitution (*Escherichia coli* numbering), known to be associated with macrolide resistance. The second one was A2062T substitution, possibly associated with pristinamycin resistance, as increased pristinamycin MICs were reported for *in vitro Mycoplasma pneumoniae* strains harboring substitutions at A2062 position.[7] Amplification and sequencing of the *parC* gene didn't show any mutation associated with fluoroquinolone resistance.

The patient initially refused moxifloxacin despite his persistent symptoms, fearing a side effect. He finally accepted a sequential treatment with doxycycline 200 mg daily for 7 days followed by moxifloxacin 400 mg daily for 14 days without complication. A new screening of resistance mutations performed during the moxifloxacin course found the previous A2058T mutation and a new S83I mutation in ParC, associated with fluoroquinolone resistance. The A2062T mutation was not detected anymore. He therefore received an additional sequential treatment with minocycline for 21 days followed by pristinamycin for 14 days.

His urethral symptoms slowly disappeared. MG NAAT performed as test of cure 3 months after completing the last sequential treatment was negative. The time period to obtain test of cure was due to the patient's reluctance to come back to the hospital. MG NAAT performed 10 days later remained negative, thus confirming microbial cure. Three-site NG and CT NAATs (urine,

rectal and pharyngeal sites) were all negative throughout this year, as well as HIV and syphilis serologies. All sexual intercourse was protected by condom as reported by the patient.

DISCUSSION

As illustrated here, MG urethritis treatment can become very complex due to antibiotic resistance. International guidelines now recommend doxycycline for 7 days instead of 1 g single dose azithromycine as empirical therapy for urethritis and cervicitis, to preserve macrolide susceptibility in case of undetected MG infection.[8]

For macrolide and fluoroquinolone-resistant MG strains, pristinamycin is the recommended option in Europe,[1] with a success rate averaging 75%.[9] But in case of failure, there is hardly any alternative, with the possible exception of spectinomycin[10] though unavailable in Europe or minocycline, which cured71% of cases.[11]

Sequential treatment has also been suggested, consisting of doxycycline followed by an appropriate antibiotic chosen according to the detected resistance mutations. Although doxycycline cure failed in approximately 70% of cases, it could strongly decrease the bacterial load hence substantially increase the second antibiotic's efficacy.[5] Here we successfully changed doxycycline for minocycline, which could be more effective on MG.[11]

Combination therapy may also be an alternative for treating highly resistant MG. Doyle et *al*. reported 75% of therapeutic success with doxycycline and pristinamycine combination therapy in 73 macrolide-resistant MG cases.[11] Durukan et *al*. reported 11/12 cured MG infections using doxycycline and sitafloxacine combination therapy, after pristinamycin failure.[12] However, sitafloxacine is not available in France.

Importantly, the first part of sequential treatment gives time to perform a resistance genotypic test, which requires a specialized molecular biology platform and takes several days. Since resistance may vary depending on antibiotic pressure, we demonstrated here the relevance of

repeating the resistance screening. We described the A2062T mutation, possibly associated with resistance to pristinamycin. Although mutations at 2062 position are not detected by any commercial assays, the A2062T mutation has been reported a few times in MG, using 23S rRNA amplification and sequencing.[6,13] So far, this mutation has never been associated with clinical resistance to pristinamycin in MG. However, we suggest it may be associated with pristinamycin resistance, as the A2062G substitution is associated with increased pristinamycin MICs for *M. pneumoniae*, the phylogenetically closest *Mycoplasma* species.[7] Considering the difficulties to grow MG, more studies focusing on pristinamycin treatment failure with characterization of 23S rRNA mutations by Sanger sequencing are needed to confirm this hypothesis.

In conclusion, we assume that the A2062T mutation is associated with pristinamycin resistance in MG. We believe that the resistance profile of MG may vary over time, depending on the different antibiotics received, and that antibiotic efficacy also depends on the bacterial load. Repeated screening for genotypic resistance, combined with the use of antibiotics with the highest antibacterial activity, could succeed in curing multidrug-resistant MG infections.

TABLE. Therapeutic management and microbiological outcomes of the MG urethritis.

Date	Day	Genito-urinary symptoms	Treatment	MG NAAT[a]	MG resistance mutations
08/23/2019	D0	Yes	Cefixime 200 mg 2/d 7 days AND azithromycin 1 g 1 day		
09/04/2019	D12	Yes		Positive	
09/07/2019	D15	Yes	Moxifloxacin 400 mg 1/d 2 days* THEN doxycycline 100 mg 2/d 7 days THEN pristinamycin 1 g 4/d 14 days		
10/11/2019	D49	Yes		Positive	A2058T[b] A2062T[c]
05/12/2020	D243	Yes	Doxycycline 100 mg 2/d 7 days THEN moxifloxacin 400 mg 1/d 14 days		
05/30/2020	D281	Yes		Positive	A2058T[b] S83I[d]
06/04/2020	D286	Yes	Minocycline 100 mg 2/d 21 days THEN pristinamycin 1 g 4/d 14 days		
09/10/2020	D384	No		Negative	
09/21/2020	D395	No		Negative	

NOTES. * Moxifloxacin was stopped due to tendon pain. a. NAAT, nucleic acid amplification test. b. Mutation in 23S rRNA associated with resistance to macrolides, *Escherichia coli* numbering. c. Mutation in 23S rRNA possibly associated with resistance to pristinamycin, *Escherichia coli* numbering. d. Mutation in ParC associated with resistance to fluoroquinolones, *Mycoplasma genitalium* numbering.

DECLARATION OF CONFICTING INTEREST

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