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Molecular Mechanisms of Solithromycin Resistance in *Mycoplasma genitalium*

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Abstract

Introduction
The cure-rate after treatment of *Mycoplasma genitalium* with a 1 g single dose of azithromycin has been decreasing from nearly 90% to 40% in recent trials. Moxifloxacin is currently the only second-line drug, but multi-drug resistant strains of *M. genitalium* are emerging. In this study, we evaluated the in vitro activity of the new fluoroketolide, solithromycin, and characterised the genetic basis for solithromycin resistance.
Methods
A collection of 40 *M. genitalium* isolates were tested; 15 strains were macrolide resistant with MIC >16 mg/L for erythromycin. MICs of solithromycin, azithromycin, erythromycin, doxycycline, ciprofloxacin, and moxifloxacin were determined by adding a defined inoculum into a Vero-cell culture with dilutions of antibiotic. Growth of *M. genitalium* was determined by quantitative PCR and MIC was defined as the minimal concentration causing 99% inhibition of growth.
Results
The MIC range of solithromycin was ≤ 0.001 - 16 mg/L (MIC_{90}: 2 mg/L). Macrolide susceptible strains had MIC_{90}: ≤0.001 mg/L whereas macrolide resistant strains had MIC_{90}: 4 mg/L. Eight strains with a 23S rRNA gene A2059G mutation (*E. coli* numbering) had a lower median MIC than that of five strains with A2058G mutations (p=0.02) and of two strains with the rare A2058C mutation (p=0.04). Solithromycin activity was significantly superior to azithromycin (p<0.0001). All strains, regardless of macrolide susceptibility carried a 23S rRNA gene with a C in position 752 in contrast to the A752 in susceptible *E. coli* strains. No other mutations were found in the region. In the 2609 position known to interact with position 752, all strains had T2609 as in susceptible *E. coli*. No mutations in ribosomal proteins L4 and L22 explaining the elevated MICs could be identified
Conclusions
The activity of solithromycin was superior to that of azithromycin, erythromycin, quinolones and doxycycline. Mutations in position 2058 lead to higher solithromycin MICs than those in position 2059 and were the only changes explaining solithromycin resistance. In Denmark, 40% of *M. genitalium* strains are azithromycin resistant but our findings suggests that 85% of these resistant strains or 94% of all *M. genitalium* strains would be susceptible to solithromycin.

Introduction

Mycoplasma genitalium is a well-established and important etiological agent of sexually transmitted infections (STIs). The cure-rate after treatment with a 1 g single dose azithromycin has been decreasing from nearly 90% in populations without macrolide resistance (Taylor-Robinson & Jensen, 2011) to 40% in the most recent controlled clinical trial (Manhart *et al*., 2013). High-level macrolide resistance is readily induced in the 5-10% of cases with susceptible strains where eradication fails. Despite apparent in vitro susceptibility to doxycycline of most strains, this antimicrobial eradicates less than 30% of the infections. Moxifloxacin is currently the only second-line drug, but multi-drug resistant strains of *M. genitalium* are emerging. In this study, we evaluated the in vitro activity of the new fluoroketolide solithromycin, and compared it to other antimicrobials currently used for treatment of *M. genitalium* infections. Furthermore, the genetic basis for solithromycin resistance was elucidated.

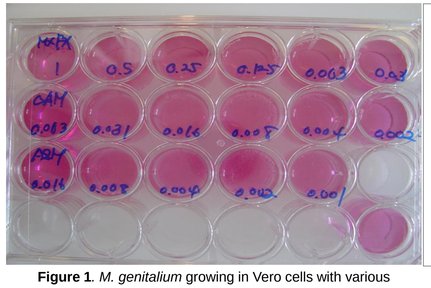


Figure 1. *M. genitalium* growing in Vero cells with various dilutions of antibiotic

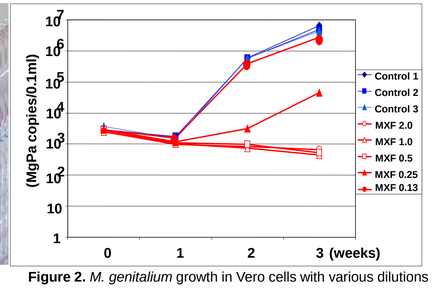


Figure 2. *M. genitalium* growth in Vero cells with various dilutions of antibiotic determined by quantitative PCR

Methods

***M. genitalium* strains**
A collection of 40 *M. genitalium* isolates originating from 38 patients were tested. These included the *M. genitalium* G37 type-strain, an early passage of the M30 strain isolated by David Taylor-Robinson in 1980 (Tully *et al.*, 1983), and one isolate kindly provided by Pat Totten, Seattle, USA. The remaining 37 strains were isolated in Copenhagen, Denmark, but the urogenital samples were obtained in seven different countries. Macrolide resistance with mutations in position 2058 or 2059 (*E. coli* numbering) and MIC >16 mg/L for erythromycin was found in 15 strains. The geographical origin of the strains is shown in Table 1.

Table 1
Distribution of *M. genitalium* strains according to country of origin and macrolide resistance.

Country of origin	Number of strains	Number of macrolide resistant strains
Sweden	12	3
Denmark	7	1
Australia	7	6
Norway	4	4
Japan	4	0
France	3	0
United Kingdom	2	0
USA	1	1

Determination of minimum inhibitory concentration (MIC)
MICs of solithromycin, azithromycin, erythromycin, doxycycline, ciprofloxacin, and moxifloxacin were determined by inoculating 2500 genome equivalents (geq) by quantitative PCR into a Vero-cell culture containing two-fold dilutions of test-antibiotic (Hamasuna *et al.*, 2005) (Fig. 1). After a three-week incubation period, cells and supernatant were harvested and growth of *M. genitalium* was determined by quantitative PCR. MIC was expressed as the minimal concentration of the test-antibiotic causing a 99% inhibition of growth when compared to the mean of the control cultures grown without antibiotic (Fig. 2).
Characterization of resistance mutations
Fragments of the 23S rRNA gene spanning positions 616-905 and 1847-2711 as well as the complete ribosomal protein L4 and L22 genes were PCR amplified and sequenced using conventional Sanger sequencing.

Table 2.
MIC of 25 macrolide susceptible and 15 macrolide resistant strains of *M. genitalium*

Antibiotic	MIC_{50} mg/L	MIC_{90} mg/L	MIC range mg/L
Solithromycin	0.001	2	≤0.001-16
Azithromycin	0.008	>16	0.002->64
Doxycycline	0.25	1	0.06-2
Ciprofloxacin	2	16	0.5 ->16
Moxifloxacin	0.125	4	0.032 ->16

Results

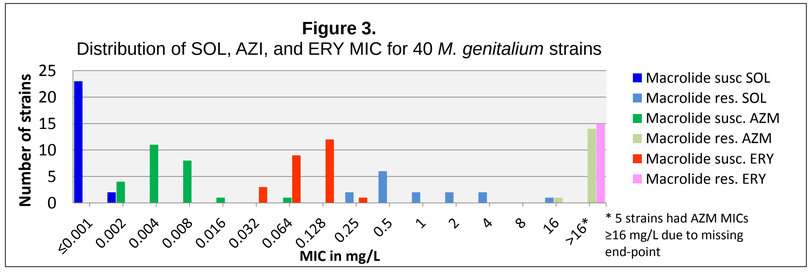
The MIC range of solithromycin was ≤ 0.001-16 mg/L (MIC_{90}: 2 mg/L). Macrolide susceptible strains had MIC_{90}: ≤0.001 mg/L whereas macrolide resistant strains had MIC_{90}: 4 mg/L. Eight strains with a 23S rRNA gene A2059G mutation (*E. coli* numbering) had a lower median MIC than that of five strains with A2058G mutations (p=0.02) and of two strains with the rare A2058C mutation (p=0.04). Solithromycin (SOL) activity was significantly superior to azithromycin (AZM) (p<0.0001) and erythromycin (ERY) (p<0.0001) in macrolide resistant and susceptible strains (Fig 3) as well as to doxycycline and the two quinolones tested.

All strains, regardless of macrolide susceptibility carried a 23S rRNA gene with a C in position 752 in contrast to the A752 in susceptible *E. coli* strains. No other mutations were found in the region. In the 2609 position known to interact with position 752, all strains had T2609 as found in susceptible *E. coli*.

Ribosomal protein L22 was remarkably conserved with 37 strains having identical amino acid (aa) sequences and the remaining three having single aa substitutions outside of the L22 loop; one strain was macrolide susceptible and two had solithromycin MICs of 0.5 mg/L.

In ribosomal protein L4, four strains with A2059G 23S rRNA gene mutations had the His69 (corresponding to the *E. coli* Gly64) amino acid substituted with Arg in the loop structure. Three of these strains had solithromycin MICs of 0.5 mg/L and one had an MIC of 1 mg/L; thus, these mutations did not appear to increase the MIC over that of other strains with similar 23S rRNA gene mutations. No other amino acid changes were found in the L4 loop structure.

Figure 3.
Distribution of SOL, AZI, and ERY MIC for 40 *M. genitalium* strains



Conclusions

The activity of solithromycin was superior to that of azithromycin, erythromycin, quinolones and doxycycline. Mutations in position 2058 lead to higher solithromycin MICs than those in position 2059 and were the only changes explaining solithromycin resistance. In Denmark, 40% of *M. genitalium* strains are azithromycin resistant but our findings suggests that 85% of these resistant strains or 94% of all *M. genitalium* strains would be susceptible to solithromycin.

References

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