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Short Communication

Prevalence of macrolide- and fluoroquinolone-resistant *Mycoplasma genitalium* strains in clinical specimens from men who have sex with men of two sexually transmitted infection practices in Berlin, Germany

Roger Dumke[a,*], Thomas Ziegler[b], Naghmeh Abbasi-Boroudjeni[b], Marcos Rust[c], Tobias Glaunsinger[d]

[a] TU Dresden, Institut für Medizinische Mikrobiologie und Hygiene, Fetscherstrasse 74, 01307 Dresden, Germany
[b] Institut für Medizinische Diagnostik Berlin MVZ, Nicolaistrasse 22, 12247 Berlin, Germany
[c] Infektiologie Ärztezentrum Seestrasse, Seestrasse 64, 13347 Berlin, Germany
[d] Praxis Prenzlauer Berg, Danziger Strasse 78b, 10405 Berlin, Germany

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ABSTRACT

Objectives: The cell-wall-less Mollicutes species *Mycoplasma genitalium* is a sexually transmitted microorganism that causes different male and female genital tract infections. In recent years, resistance of the pathogen to macrolides and fluoroquinolones has been increasingly reported worldwide and is more frequent in risk groups.
Methods: To determine the rates of antimicrobial resistance, *M. genitalium* strains in 195 specimens from 154 outpatients (154 first and 41 follow-up samples) treated in two specialised practices between September 2017 and December 2018 in Berlin, Germany, were analysed.
Results: The included patients were predominantly men who have sex with men (MSM) (91.6%) and were HIV-positive in many cases (49.4%). Only 27.3% of *M. genitalium*-positive patients reported symptoms. Among the first samples (mainly rectal swabs) (57.8%), mutations associated with macrolide (23S rRNA) and quinolone (*parC* gene) resistance were detected in 79.9% and 13.0% of strains, respectively. Resistance to both classes of antibiotics was found in 11.7% of specimens. Changes of A → G at position 2072 of 23S rRNA and of serine at position 83 of ParC were the most frequent alterations.
Conclusion: Although azithromycin is recommended as a first-line antibiotic to treat infections with *M. genitalium* in MSM, according to these data its use must be highly limited in Berlin. Besides the need for resistance studies regarding strains circulating in other locations and among different patient groups in Germany, the results emphasise the importance of intensified antibiotic resistance testing of *M. genitalium* to avoid a further increase in treatment failures in infections with this emerging human pathogen.

1. Introduction

Mycoplasma genitalium is a sexually transmitted cell-wall-less bacterium first isolated from urethritis patients in 1980. The microorganism was identified as a cause of non-gonococcal urethritis in men and of cervicitis in women and is associated with female reproductive tract and pelvic inflammatory disease [1,2]. The prevalence of *M. genitalium* in the general population has been found to vary between 1–3% and is increasing among risk patients (men who have sex with men (MSM), sex workers, human immunodeficiency virus (HIV)-positive patients [3]). Epidemiological investigations are hampered by the frequently asymptomatic infections and the difficulty of cultivating *M. genitalium* [1]. Furthermore, treatment of infected patients is challenging as members of the Mollicutes group are intrinsically resistant to β-lactam antibiotics. Tetracyclines show excellent in vitro activity but limited in vivo effectiveness (approximately 30–35%). Azithromycin treatment is widely recommended as first-line therapy and leads to cure rates of 80–88% [4,5]. In recent years, treatment failures have increased greatly in many countries. Molecular characterisation of isolates from these patients shows mutations in region V of the single copy of 23S rRNA of *M. genitalium* (position 2071 or 2072, corresponding to positions 2058 and 2059 in *Escherichia coli*). Both transitions cause high-level resistance to

* Corresponding author.
E-mail address: roger.dumke@tu-dresden.de (R. Dumke).

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azithromycin. Quinolones (moxifloxacin) have been successfully used as second-line antibiotics in cases of treatment failure. Unfortunately, the number of patients without response to treatment is also rising (up to 30% [5]) and analysis of strains showed mutations in the quinolone resistance-determining region (QRDR) of the *parC* gene of *M. genitalium*. In addition, alterations in the gyrase A (*gyrA*) gene have been detected but their role in resistance remains unclear [6]. Descriptions of multidrug-resistant strains (combined macrolide and quinolone resistance) and the limited repertoire of well-tested, effective and approved therapy alternatives increase concern regarding untreatable *M. genitalium* infections [1].

Apart from one report that characterised a small number of samples [7], there is no information about the resistance pattern of *M. genitalium* strains circulating in Germany. This is especially the case for risk populations. As in many other countries, testing for *M. genitalium* is not usually recommended in routine screening procedures for sexually transmitted infections (STIs). This study aimed to assess the resistance pattern of *M. genitalium* strains among outpatients in two practices in Berlin, Germany.

2. Patients and methods

The specimens investigated in this study were collected between September 2017 and December 2018 as part of extended monitoring of STIs in patients belonging to risk populations in two practices specialised in the treatment of these diseases. Duplicate samples from the same patient were excluded. DNA from the samples was isolated using an EZ1 DNA Tissue Kit and EZ1 Advanced XL automated extraction system (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. *M. genitalium* was detected by real-time PCR with a commercial Anyplex™ STI-5 Detection Assay (Seegene, Seoul, South Korea) on a CFX96™ Cycler (Bio-Rad, Hercules, CA) and the data were analysed by end point-catcher melting temperature analysis using the Seegene Viewer program following the manufacturer's guidelines. DNA of positive samples was stored frozen until molecular analysis of resistance (within 4 weeks after sampling).

During the investigation period, 154 first samples (89 rectal swabs, 60 first-void urines, 3 vaginal swabs, 1 urethral swab and 1 pharyngeal swab) tested positive for *M. genitalium*. Resistance-associated mutations (RAMs) in 23S rRNA, *parC* and *gyrA* of these strains were detected as previously described [7]. Briefly, parts of genes in which RAMs are located were amplified using the primers summarised in Supplementary Table S1 and the amplicons were sequenced. The obtained sequences were aligned against corresponding parts of the *M. genitalium* reference strain G37 (GenBank accession no. **NC_000908.2**). In 26.6% of patients (41/154) who tested *M. genitalium*-positive in the first specimen, the follow-up sample (mean interval between both samples, 50 days; range, 3–324 days) was positive. Strains in these specimens were analysed for mutations and were compared with the strain in the first sample.

Table 1
Characteristics of patients testing positive for *Mycoplasma genitalium* ($n = 154$).

Characteristic	%[a]
Age (years) [mean (range)]	36.3 (20–61)
Male	98.1
MSM	91.6
HIV-positive	49.4
Urethral symptoms	19.5
Anorectal symptoms	7.8

MSM, men who have sex with men; HIV, human immunodeficiency virus.
[a] Data are percentage unless otherwise stated.

3. Results

Table 1 summarises the main characteristics of the patients included in the study. *M. genitalium* was detected in first samples from 151 men (98.1%) and 3 women (1.9%). Among these patients, 91.6% of men identified themselves as MSM and 49.4% are HIV-positive. Urogenital and/or anorectal symptoms (pain, pruritus, discharge) were reported in only 19.5% and 7.8% of cases. Genetic resistance data were available for all 154 first *M. genitalium*-positive specimens (Table 2). Macrolide RAMs were found in 123 samples (79.9% of all strains tested). Among them, the A→G transition at position 2072 was most frequent [78.0% (96/123) of macrolide-resistant strains], followed by A→G mutation at position 2071 (15.4%; 19/123). In 6.5% (8/123) of samples a change from A→T at position 2071 was detected. The QRDR of the *parC* gene of 20 strains (13.0%) contained mutations resulting in amino acid changes in topoisomerase IV that are suspected to cause fluoroquinolone resistance. In 80.0% (16/20) of quinolone-resistant

Table 2
Macrolide resistance-associated mutations (RAMs) in 23S rRNA and fluoroquinolone RAMs in *parC* with the corresponding amino acid changes in *Mycoplasma genitalium* strains ($n = 154$).

Resistance	Gene(s)	Mutation (amino acid change)[a]	No. (%) of samples
Macrolides	23S rRNA	A2072G	96 (62.3)
		A2071G	19 (12.3)
		A2071T	8 (5.2)
		Total	123 (79.9)
Fluoroquinolones	*parC*	G248T (S83I)	12 (7.8)
		G248A (S83N)	4 (2.6)
		G259T (D87Y)	2 (1.3)
		A260G (D87G)	1 (0.6)
		G259A (D87N)	1 (0.6)
		Total	20 (13.0)
Macrolides and fluoroquinolones	23S rRNA + *parC*	A2071G + G248T (S83I)	5 (3.2)
		A2072G + G248T (S83I)	4 (2.6)
		A2072 G + G248A (S83N)	2 (1.3)
		A2071T + G248T (S83I)	2 (1.3)
		A2071G + G259T (D87Y)	1 (0.6)
		A2072G + G259T (D87Y)	1 (0.6)
		A2072G + G259A (D87N)	1 (0.6)
		A2072G + A260G (D87G)	1 (0.6)
		A2071G + G248A (S83N)	1 (0.6)
		Total	18 (11.7)

[a] Positions are given according to the *M. genitalium* G37 genome (GenBank accession no. **NC_000908.2**).

Table 3
Characterisation of the resistance pattern of *Mycoplasma genitalium*-positive follow-up samples ($n = 41$).

Difference(s) between strains in first and follow-up sample	No. (%) of isolates
None (both wild-type)	2 (4.9)
None (both macrolide-resistant)	23 (56.1)
None (both macrolide- and fluoroquinolone-resistant)	7 (17.1)
Change from wild-type to macrolide-resistant	2 (4.9)
Change of macrolide RAM	3 (7.3)
Change of macrolide RAM and occurrence of fluoroquinolone resistance	3 (7.3)
Change of macrolide RAM and loss of fluoroquinolone resistance	1 (2.4)

M. genitalium, changes of serine at position 83 to isoleucine or asparagine were found. In the remaining four samples, three different transitions of aspartic acid at position 87 of ParC were detected. Two strains (1.3% of all *M. genitalium*-positive samples) showed a quinolone RAM without changes in the resistance-relevant part of 23S rRNA. In 18 strains (11.7%), resistance to macrolides and fluoroquinolones can be assumed. These strains demonstrate a broad spectrum of combinations of specific mutations both in 23S rRNA and *parC* without a clear predominance of a particular genotype. Furthermore, sequencing of the QRDR in the *gyrA* gene yielded wild-type sequences in all strains. Outside of the QRDR, mutations of G332A (resulting in a change from arginine to lysine at position 111 of GyrA) or G356A (glycine to asparagine at position 119) were found in one sample each.

In 83.8% of patients with a *M. genitalium*-positive test result, a follow-up sample was obtained (in most cases to test for cure after antibiotic treatment). After comparing the *M. genitalium*-positive follow-up specimens ($n = 41$) (Table 3) with the first samples, most strains (78.0%; 32/41) showed identical RAMs or wild-type sequences both in 23S rRNA and *parC*. These were mainly mutations of 23S rRNA (56%). Seven patients carried a strain with double resistance. Failure of macrolide treatment in susceptible *M. genitalium* can be excluded as azithromycin was not administered between samplings to either of the patients with strains showing wild-type-specific sequences in the first and follow-up specimen. In contrast, development of macrolide resistance during antibiotic treatment is probable in two patients. This hypothesis is supported by the fact that samples from the same location (urethra) were investigated in both cases and the corresponding strains showed the same genotype according to the variable number of tandem repeats (VNTR) in *MG_309* and single nucleotide polymorphisms (SNPs) in *MG_191* genes (data not shown [7]). Between samplings (time between treatment and sampling, 18 days and 31 days, respectively), these two patients were treated with a 5-day regimen of azithromycin. In seven samples (17.1%), the pattern of RAMs in comparison with the first sample suggested re-infection with a different strain during the interval between samplings or colonisation with two genotypes of *M. genitalium*. The sample location was identical in five of these patients (two urethra and three rectum) but differed in the remaining two men. No induction of quinolone resistance after treatment with moxifloxacin (400 mg for 10 days) was observed. However, in one of these three patients, ongoing colonisation with a quinolone-susceptible strain could not be excluded (time between moxifloxacin treatment and sampling, 97 days, both were urine samples, same genotype according to partial sequence of *MG_309* and *MG_191*, respectively).

4. Discussion

This retrospective study analysed macrolide and quinolone RAMs of *M. genitalium* strains from 154 outpatients in Berlin. In agreement with previous reports, the results confirmed a high percentage of asymptomatic carriers [8] as the majority of *M. genitalium*-positive patients (approximately 73%) reported a lack of urogenital or anorectal disorders. Most patients included in the study belong to risk groups for acquiring *M. genitalium* infections (MSM and/or HIV-positive). In a systematic review, prevalence rates between 2% and 6% in MSM were noted [3], but significantly higher rates up to 17% have been calculated in more recent reports [9]. Because of the high proportion of MSM (91.6%), >50% of specimens tested in this study were rectal swabs. In strains of the small number of samples from patients who probably do not belong to the MSM group and are not HIV-positive ($n = 11$), macrolide, quinolone or combined resistance was found in only one sample each, indicating differences in the prevalence of resistance between patient groups.

Whereas rates of macrolide resistance among *M. genitalium* circulating in the general population are usually <50% [5], the measured rate of macrolide-resistant strains in the present report (79.9%) is typical for risk groups in urban regions. Recent studies measured rates between 70.6% and 84.2% among MSM in the USA, Ireland, Spain and Australia [9–13]. Azithromycin therapy of confirmed *M. genitalium* infection for an extended period of 5 days is recommended in the current European guideline [8] and was used to treat most of the patients in the present study. In the presence of susceptible strains, the mean efficacy of this therapeutic regimen has been calculated to be 88% [4]. Because of the high rate of macrolide-resistant strains in the first specimen, estimation of treatment failures is difficult. For two strains demonstrating wild-type sequences of 23S rRNA in the first specimen, follow-up samples showed macrolide resistance following azithromycin treatment. Regarding one strain, sequencing results clearly demonstrated a mixture of wild-type and mutated genotype, which is also reported in a few cases in other studies [14]. According to the data from the current study, azithromycin can no longer be recommended for the treatment of *M. genitalium* infections among MSM in Berlin without resistance testing of strains. As significant differences between location, sex and patient groups are described [15], further studies are strongly recommended in other regions of Germany and within different populations to get a more comprehensive overview of the resistance situation.

Despite their cost and current discussions about adverse effects [16], the importance of fluoroquinolones as a treatment option in *M. genitalium* infections is increasing. Owing to the high rate of macrolide-resistant strains, this is especially the case among risk populations. The detected rate of quinolone resistance of 13.0% is relatively high in comparison with data from the general population in other European countries [1] but is not surprising for strains among MSM. In this group, quinolone resistance rates up to 30% were found [10,11]. Consistent with other reports, amino acid changes concerned mainly aspartic acid at position 87 and serine at position 83 of ParC. In a recent study, the latter transition was linked with a significant increase in minimum inhibitory concentrations (MICs) to different quinolones [6]. Interestingly, mutations in the QRDR of gyrase A of *M. genitalium* were not detected in any of the 195 positive samples. This is in contrast to

other studies [6,15]. Irrespective of the discussion as to whether these transitions influence susceptibility to quinolones [6], alterations of GyrA can be assumed not to contribute to resistance within the investigated population, which is consistent with a recent report from England [14]. It should further be noted that all strains showing mutations associated with quinolone resistance were found in the first sample or in follow-up samples in which the resistance pattern makes re-infection probable.

These results indicate that there is an urgent need for molecular antimicrobial testing of *M. genitalium* strains prior to antibiotic therapy. Regarding follow-up samples, the current European guideline recommends use of an assay detecting macrolide resistance [8]. Whereas different commercial and in-house tests for combined detection of *M. genitalium* and macrolide RAMs are available [17,18], quinolone resistance currently has to be confirmed by amplification and sequencing of the QRDR of *parC*. A recent report from Japan described different mutations in the *parC* gene of *M. genitalium* strains sampled between 2007 and 2017 that cause changes of 11 amino acids in the region from positions 62–119 of the QRDR of ParC [6]. Further studies will have to elucidate whether all of these transitions influence susceptibility to quinolones. For example, amino acid change S83N appears not to be associated with increased moxifloxacin MICs [19]. However, increasing use of moxifloxacin will require laboratories that are able to detect resistance in *M. genitalium*. This is important especially for patients carrying strains with dual resistance to both macrolides and quinolones. With 12% of samples in the present study, the rate is at a level that demands attention. Therapeutic alternatives (doxycycline or pristinamycin [8]) are of limited clinical efficacy [4] or are not approved in all countries.

To our knowledge, this is the first study in Germany reporting the prevalence of macrolide and fluoroquinolone resistance of *M. genitalium* strains among patients belonging to risk groups for acquiring and carrying this infection. In comparison with the international situation, the rate of macrolide resistance is relatively high and quinolone resistance is at a critical level. Besides the efforts to develop more effective therapeutic regimens and new antibiotic alternatives to treat infected patients, current testing and guidelines should be modified to ensure adequate treatment of infections in risk populations. This includes the implementation of combined diagnostic and resistance testing for more guided treatment of patients with confirmed *M. genitalium* infection [20] in order to reduce the transmission of this increasingly recognised pathogen. However, keeping in mind the rates of macrolide and quinolone resistance, the possibility of antibiotic treatment-induced resistance, the low rates of symptomatic infections and the mostly mild clinical manifestations, the uncritical screening of asymptomatic MSM should be questioned.

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Competing interests

None declared.

Ethical approval

This retrospective study was approved by the Institutional Review Board of TU Dresden (Dresden, Germany) [no. EK 473122017]. Patients provided informed consent before participating.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.06.015.

References

[1] Unemo M, Jensen JS. Antimicrobial-resistant sexually transmitted infections: gonorrhoea and *Mycoplasma genitalium*. Nat Rev Urol 2017;14:139–52.
[2] Lis R, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* infection and female reproductive tract disease: a meta-analysis. Clin Infect Dis 2015;61:418–26.
[3] Baumann L, Cina M, Egli-Gany D, Goutaki M, Halbeisen FS, Lohrer GR, et al. Prevalence of *Mycoplasma genitalium* in different population groups: systematic review and meta-analysis. Sex Transm Infect 2018;94:255–62.
[4] Bradshaw CS, Jensen JS, Waites KB. New horizons in *Mycoplasma genitalium* treatment. J Infect Dis 2017;216(Suppl. 2):S412–9.
[5] Manhart LE, Jensen JS, Bradshaw CS, Golden MR, Martin DH. Efficacy of antimicrobial therapy for *Mycoplasma genitalium* infections. Clin Infect Dis 2015;61(Suppl. 8):S802–17.
[6] Hamasuna R, Le PT, Kutsuna S, Furubayashi K, Matsumoto M, Ohmagari N, et al. Mutations in ParC and GyrA of moxifloxacin-resistant and susceptible *Mycoplasma genitalium* strains. PLoS One 2018;13:e0198355.
[7] Dumke R, Thürmer A, Jacobs E. Emergence of *Mycoplasma genitalium* strains showing mutations associated with macrolide and fluoroquinolone resistance in the region Dresden, Germany. Diagn Microbiol Infect Dis 2016;86:221–3.
[8] Jensen JS, Cusini M, Gomberg M, Moi H. 2016 European guideline on *Mycoplasma genitalium* infections. J Eur Acad Dermatol Venereol 2016;30:1650–6.
[9] Dionne-Odom J, Geisler WM, Aaron KJ, Waites KB, Westfall AO, van der Pol B, et al. High prevalence of multidrug-resistant *Mycoplasma genitalium* in human immunodeficiency virus-infected men who have sex with men in Alabama. Clin Infect Dis 2018;66:796–8.
[10] Mulligan V, Lynagh Y, Clarke S, Unemo M, Crowley B. Prevalence, macrolide resistance, and fluoroquinolone resistance in *Mycoplasma genitalium* in men who have sex with men attending an sexually transmitted disease clinic in Dublin, Ireland in 2017–2018. Sex Transm Dis 2019;46:e35–7, doi:http://dx.doi.org/10.1097/OLQ.0000000000000940.
[11] Barberá MJ, Fernández-Huerta M, Jensen JS, Caballero E, Andreu A. *Mycoplasma genitalium* macrolide and fluoroquinolone resistance: prevalence and risk factors among a 2013–2014 cohort of patients in Barcelona, Spain. Sex Transm Dis 2017;44:457–62.
[12] Read TRH, Murray GL, Danielewski JA, Fairley CK, Doyle M, Worthington K, et al. Symptoms, sites, and significance of *Mycoplasma genitalium* in men who have sex with men. Emerg Infect Dis 2019;25:719–27.
[13] Couldwell DL, Jalocon D, Power M, Jeoffreys NJ, Chen SC, Lewis DA. *Mycoplasma genitalium*: high prevalence of resistance to macrolides and frequent anorectal infection in men who have sex with men in western Sydney. Sex Transm Infect 2018;94:406–10.
[14] Pitt R, Fifer H, Woodford N, Alexander S. Detection of markers predictive of macrolide and fluoroquinolone resistance in *Mycoplasma genitalium* from patients attending sexual health services in England. Sex Transm Infect 2018;94:9–13.
[15] Sweeney EL, Trembizki E, Bletchly C, Bradshaw CS, Menon A, Francis F, et al. Levels of *Mycoplasma genitalium* antimicrobial resistance differ by both region and gender in the State of Queensland, Australia: implications for treatment guidelines. J Clin Microbiol 2019;57: e01555-18.
[16] Tandan M, Cormican M, Vellinga A. Adverse events of fluoroquinolones vs. other antimicrobials prescribed in primary care: a systematic review and meta-analysis of randomized controlled trials. Int J Antimicrob Agents 2018;52:529–40.
[17] Tabrizi SN, Su J, Bradshaw CS, Fairley CK, Walker S, Tan LY, et al. Prospective evaluation of ResistancePlus MG, a new multiplex quantitative PCR assay for detection of *Mycoplasma genitalium* and macrolide resistance. J Clin Microbiol 2017;55:1915–9.
[18] Gossé M, Lysvand H, Pukstad B, Nordbø SA. A novel simple probe PCR assay for detection of mutations in the 23S rRNA gene associated with macrolide resistance in *Mycoplasma genitalium* in clinical samples. J Clin Microbiol 2016;54:2563–7.
[19] Unemo M, Salado-Rasmussen K, Hansen M, Olsen AO, Falk M, Golparian D, et al. Clinical and analytical evaluation of the new Aptima *Mycoplasma genitalium* assay, with data on *M. genitalium* prevalence and antimicrobial resistance in *M. genitalium* in Denmark, Norway and Sweden in 2016. Clin Microbiol Infect 2018;24:533–9.
[20] Read TRH, Fairley CK, Murray GL, Jensen JS, Danielewski J, Worthington K, et al. Outcomes of resistance-guided sequential treatment of *Mycoplasma genitalium* infections: a prospective evaluation. Clin Infect Dis 2019;68:554–60.