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Remarkable increase in fluoroquinolone-resistant *Mycoplasma genitalium* in Japan

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Objectives: We determined the prevalence of macrolide and fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium* DNA specimens from men with non-gonococcal urethritis (NGU) and analysed their effects on antibiotic treatments of *M. genitalium* infections.

Methods: In this retrospective study, we examined antibiotic resistance-associated mutations in the 23S rRNA, *gyrA* and *parC* genes of *M. genitalium* and the association of the mutations with microbiological outcomes of antibiotic treatments in men with *M. genitalium*-positive NGU.

Results: No macrolide resistance-associated mutations in the 23S rRNA gene were observed in 27 *M. genitalium* DNA specimens in 2011 and in 24 in 2012. However, 5 of 17 in 2013 had 23S rRNA mutations. Three of 15 in 2011, 6 of 19 in 2012 and 8 of 17 in 2013 had fluoroquinolone resistance-associated alterations in ParC. Three in 2013 had both the antibiotic resistance-associated alterations coincidentally. In two men with *M. genitalium* harbouring 23S rRNA mutations, the mycoplasma persisted after treatment with a regimen of 2 g of extended-release azithromycin (AZM-SR) once daily for 1 day. All nine men with mycoplasma harbouring ParC alterations were microbiologically cured with a regimen of 100 mg of sitafloxacin twice daily for 7 days.

Conclusions: Macrolide- or fluoroquinolone-resistant *M. genitalium* appears to be increasing, and the increase in fluoroquinolone-resistant mycoplasmas is especially remarkable in Japan. Mycoplasmas harbouring 23S rRNA mutations would be resistant to the AZM-SR regimen, but those harbouring ParC alterations would still be susceptible to the sitafloxacin regimen.

Keywords: GyrA, ParC, 23S rRNA, sitafloxacin, azithromycin

Introduction

Recently, *Mycoplasma genitalium* has been shown to be significantly associated with non-gonococcal urethritis (NGU) in men and with cervicitis, endometritis, salpingitis and pelvic inflammatory diseases in women.[1] For treatment of *M. genitalium* infections, a single 1 g dose of azithromycin has been considered as one of several relevant treatments.[2] However, azithromycin treatment failure was reported in cases of *M. genitalium*-positive NGU, and macrolide-resistant clinical strains of *M. genitalium* were isolated from some patients with treatment failure.[3,4] In these strains, mutations were found in the residues of the 23S rRNA gene of *M. genitalium* corresponding to A-2058 and A-2059 in region V of the 23S rRNA gene of *Escherichia coli*, which are critical for the binding of macrolides.[5] These mutations could confer a high level of resistance to azithromycin because this species possesses only one copy of the rRNA gene operon.[6] Several studies, including our previous study, have reported that a single-dose regimen of 1 g of azithromycin selects mutants harbouring mutations in the 23S rRNA gene.[3,7,8] In Japan, extended regimens of azithromycin are not approved for the treatment of urogenital infections, but a regimen of extended-release azithromycin (AZM-SR) at 2 g once daily for 1 day is available clinically. This regimen provided total azithromycin exposures in serum and leucocytes that were similar to those of the regimen of 500 mg of azithromycin once daily for 3 days, and an additional therapeutic benefit due to front-loading of the dose, which achieved significantly higher exposures in serum and leucocytes during the first

24 h after the start of therapy.[9] We have been using the AZM-SR regimen in the treatment of men with NGU in Japan.

For persistent *M. genitalium* infections unsuccessfully treated with azithromycin regimens, several studies reported that 7 and 10 day regimens of 400 mg of moxifloxacin were highly effective as second-, third- or fourth-line treatments.[10,11] Moxifloxacin is also not approved for the treatment of urogenital infections in Japan. In *in vitro* susceptibility tests, sitafloxacin was as active as moxifloxacin against *M. genitalium* strains, including reference strains and currently isolated strains.[12] We reported that the regimen of 100 mg of sitafloxacin twice daily for 7 days was highly effective against *M. genitalium* infections,[13] although treatment failure with this regimen was reported recently.[14] We have also been using the sitafloxacin regimen in the treatment of men with NGU in Japan. The central mechanism of fluoroquinolone resistance involves alterations of the GyrA subunit of DNA gyrase and/or the ParC subunit of topoisomerase IV in many bacterial species, including mycoplasmas and ureaplasmas.[15] We first detected the amino acid changes Ser-83→Asn, Asp-87→Tyr and Asp-87→Val in ParC, corresponding to changes at amino acid positions 80 and 84 in ParC of *E. coli*, in *M. genitalium* DNAs in urine specimens from men with NGU.[16] Tagg *et al.*[17] also reported an amino acid change (Met-95→Ile) in GyrA and amino acid changes at positions 83 and 84 in ParC in *M. genitalium* DNAs obtained from clinical specimens. These alterations in ParC were located within the region analogous to the quinolone resistance-determining region (QRDR) of GyrA.[18] In 2013, Couldwell *et al.*[19] reported the first cases of moxifloxacin treatment failure in men with *M. genitalium* infections and the significant association of these treatment failures with amino acid changes in the QRDR of ParC.

In the present study, we examined a portion of the 23S rRNA gene and the QRDR of the *gyrA* gene and the analogous region of the *parC* gene in *M. genitalium* DNAs taken from urine specimens of men with *M. genitalium*-positive NGU for macrolide resistance-associated mutations and for fluoroquinolone resistance-associated amino acid changes, respectively. We genotyped the mycoplasmas harbouring macrolide and/or fluoroquinolone resistance-associated alterations for their clonalities. In addition, we examined the antibiotic resistance-associated alterations in *M. genitalium* for their effects on microbiological outcomes of antibiotic treatments for *M. genitalium*-positive NGU.

Methods

Patients and urine specimens

This retrospective study was approved by the Institutional Review Board of the Graduate School of Medicine, Gifu University, Japan (reference number 22-11). We collected urine specimens from men with NGU who had visited a urological clinic (iClinic) in Sendai, Japan, between January 2011 and September 2013 for evaluation of microbial aetiologies of urethritis. All specimens had been examined for the presence of *Chlamydia trachomatis*, *M. genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum* and *Ureaplasma urealyticum* by nucleic acid amplification tests (NAATs).[20] The DNA specimens remaining after the examinations were stored at −70°C. During the study period, we collected 99 DNA specimens that were positive for *M. genitalium* regardless of the presence or absence of other bacterial species. Initially, we excluded nine specimens from patients who had taken any antibiotics for 3 months before attending the clinic. The remaining 90 specimens were included in this study.

Mutations in the 23S rRNA, gyrA and parC genes of M. genitalium

For the 90 specimens, the portion of the 23S rRNA gene, corresponding to region V of the 23S rRNA gene of *E. coli*, the region corresponding to the QRDR of the *E. coli gyrA* gene and the analogous region of the *parC* gene were amplified from the *M. genitalium* DNAs by PCR, and sequencing of the PCR products was performed as reported previously.[16,21]

Relatedness of M. genitalium strains harbouring antibiotic resistance-associated alterations

To assess the relatedness of *M. genitalium* DNAs of clinical strains harbouring antibiotic resistance-associated alterations, we genotyped their DNAs by analysing single-nucleotide polymorphisms (SNPs) in the MG191 gene and short tandem repeats (STRs) of an AGT/AAT unit in the MG309 gene in comparison with the corresponding genes in the type strain of *M. genitalium* G37 as reported previously.[22,23] The combined sequences of the MG191 and MG309 genes determined from *M. genitalium* DNA specimens harbouring mutations in 23S rRNA and/or the *parC* genes and the type strain were aligned with the multiple-alignment software in the MEGA6 program package.[24] A dendrogram was drawn to visualize the phylogenic distances among the *M. genitalium* DNA specimens with an unweighted pair-group method with arithmetic mean.

Effects of antibiotic resistance-associated alterations in M. genitalium on microbiological outcomes of antibiotic treatments for M. genitalium-positive NGU

We retrieved microbiological data from the medical records of patients with NGU in whom *M. genitalium* DNA specimens could be analysed for mutations in all of the 23S rRNA, *gyrA* and *parC* genes. At the first visit, all patients were told to re-visit the clinic for a test of cure 3 weeks later. Microbiological cure was judged to have occurred if *M. genitalium* that had been detected in first-voided urine (FVU) before treatment was examined by NAAT and eradicated within 4 weeks after the beginning of treatment. We analysed the association of the macrolide and/or fluoroquinolone resistance-associated alterations with microbiological outcomes of the antibiotic treatments. Although the patients had been told that their regular sex partners should be examined for genital infections, we did not obtain any information regarding partner management.

Results

Mutations in the 23S rRNA, gyrA and parC genes of M. genitalium

In 51 of the 90 DNA specimens examined, respective DNA fragments of the 23S rRNA, *gyrA* and *parC* genes were amplified by PCR and analysed for mutations in all of these genes. Among the remaining 39 DNA specimens, DNA fragments of only the 23S rRNA gene could be amplified and analysed in 17, but no DNA fragments of the 23S rRNA, *gyrA* and *parC* genes were amplified in 22. In 4 and 1 of the 68 specimens that could be analysed for mutations only in the 23S rRNA gene, *M. genitalium* had an A-to-G transition at nucleotide positions 2071 and 2072, respectively, in the 23S rRNA gene, corresponding to positions 2058 and 2059 in *E. coli* (Table 1). In 4 and 1 of 51 the specimens that could be analysed for the *gyrA* and *parC* genes, *M. genitalium* had a C-to-T transition at nucleotide positions 267 and 270 in the *gyrA* gene, respectively, resulting in no amino acid changes in GyrA.

Table 1. NGU patients infected with *M. genitalium* in whom the mycoplasma DNA specimens collected from their FVU had macrolide resistance-associated mutations in the 23S rRNA gene and/or fluoroquinolone resistance-associated amino acid changes in ParC

Patient	Year *M. genitalium* detected	Mutation in 23S rRNA	Amino acid change in ParC	MG309 STR copy no.	MG191 SNP type[a]	Treatment	Microbiological outcome
1	2011	WT	Ser-80→Asn	9	14	100 mg of SFX twice daily for 7 days	eradicated
2	2011	WT	Ser-80→Asn	10	14	100 mg of SFX twice daily for 7 days	eradicated
3	2011	WT	Ser-80→Asn	10	7	100 mg of SFX twice daily for 7 days	eradicated
4	2012	WT	Ser-80→Asn	8	A	2 g of AZM-SR once daily for 1 day	eradicated
5	2012	WT	Ser-80→Asn	8	B	100 mg of SFX twice daily for 7 days	eradicated
6	2012	WT	Ser-80→Ile	11	7	2 g of AZM-SR once daily for 1 day	eradicated
7	2012	WT	Asp-84→Asn	9	C	2 g of AZM-SR once daily for 1 day	unknown
8	2012	WT	Ser-80→Asn	9	14	2 g of AZM-SR once daily for 1 day	eradicated
9	2012	WT	Asp-84→Asn	10	21	2 g of AZM-SR once daily for 1 day	eradicated
10	2012	WT	Ala-116→Glu	9	7	2 g of AZM-SR once daily for 1 day	eradicated
11	2013	A-2058→G	Ser-80→Asn	11	7	2 g of AZM-SR once daily for 1 day	persistent
12	2013	WT	Ser-80→Asn	10	7	100 mg of SFX twice daily for 7 days	eradicated
13	2013	A-2058→G	Ser-80→Asn	14	7	2 g of AZM-SR once daily for 1 day	persistent
14	2013	WT	Ser-80→Ile	12	3	100 mg of SFX twice daily for 7 days	eradicated
15	2013	WT	Ser-80→Asn	12	D	100 mg of SFX twice daily for 7 days	eradicated
16	2013	A-2058→G	Ser-80→Asn	12	7	100 mg of SFX twice daily for 7 days	eradicated
17	2013	A-2058→G	WT	12	7	100 mg of SFX twice daily for 7 days	eradicated
18	2013	A-2059→G	WT	15	20	100 mg of SFX twice daily for 7 days	eradicated
19	2013	WT	Ser-80→Asn	10	E	100 mg of SFX twice daily for 7 days	eradicated
20	2013	WT	Ser-80→Ile	11	21	2 g of AZM-SR once daily for 1 day	eradicated

SFX, sitafloxacin; WT, wild-type.
The nucleotide positions in the 23S rRNA gene and the amino acid positions in ParC are identified according to *E. coli* numbering.
[a]MG191 SNP types were derived from the literature.[22] Novel types were designated A–E.

M. genitalium had a single mutation in the *parC* gene of a G-to-A transition at nucleotide position 248 (Ser-83→Asn) in 12 specimens, a G-to-T transition at position 248 (Ser-83→Ile) in 3 specimens, a G-to-A transition at position 259 (Asp-87→Asn) in 2 specimens and a C-to-A transition at position 356 (Ala-119→Glu) in 1 specimen. The Ser-83→Asn or Ile, Asp-87→Asn and Ala-119→Glu transitions corresponded to changes at amino acid positions 80, 84 and 116 in *E. coli* ParC, respectively. The amino acid changes at positions 80 and 84 were located in the QRDR in ParC. However, the Ala-116→Glu transition in ParC was located outside the region. It is uncertain whether the amino acid change Ala-116→Glu in ParC could contribute to fluoroquinolone resistance in *M. genitalium*. In this study, the amino acid change Ala-116→Glu in ParC was not involved in fluoroquinolone resistance-associated amino acid changes. In the following text and Table 1, the nucleotide positions in the 23S rRNA gene and the amino acid positions in ParC are identified according to *E. coli* numbering.

Prevalence of macrolide and/or fluoroquinolone resistance-associated alterations in clinical strains of M. genitalium

The 68 *M. genitalium* DNA specimens that could be examined for the 23S rRNA gene comprised 27 specimens collected in 2011, 24 in 2012 and 17 in 2013. The 51 specimens that could be examined for the 23S rRNA, *gyrA* and *parC* genes comprised 15 specimens collected in 2011, 19 in 2012 and 17 in 2013. No DNA specimens in which macrolide resistance-associated mutations were detected in the 23S rRNA gene of *M. genitalium* were observed in 2011 and 2012. In 2013, however, 5 of 17 specimens had the 23S rRNA mutations. In 2011, 3 of 15 specimens had the fluoroquinolone resistance-associated amino acid change Ser-80→Asn in ParC. Of 19 specimens in 2012, four and two had the amino acid changes at positions 80 and 84 in ParC, respectively. In 2013, 8 of 17 had the amino acid changes at position 80 in ParC. Of the five specimens harbouring the 23S rRNA mutations and the eight specimens harbouring the ParC alterations, three had both macrolide and fluoroquinolone resistance-associated alterations coincidentally.

Relatedness of clinical strains of M. genitalium harbouring antibiotic resistance-associated alterations

In all 20 specimens in which *M. genitalium* had macrolide and/or fluoroquinolone resistance-associated alterations, both the SNP in the MG191 gene and the STRs in the MG309 gene were determined (Table 1). Fifteen of 20 *M. genitalium* DNA specimens had five MG191 SNP types identical to those previously reported.[22–26] The remaining five sequences of the MG191 gene were designated as novel MG191 SNP types (A–E). These partial sequences in the MG191 gene were deposited in GenBank under accession numbers AB919117, AB919118, AB919119, AB919120 and AB919121.

A dendrogram was formed based on the combined genotype profiles for the MG191 SNP sequence types and the MG309 STR locus (Figure 1). Sixteen combinations of the determined sequences in the MG191 gene and the numbers of STRs in the MG309 gene were observed. The *M. genitalium* DNA specimens from Patients 1, 2 and 8 had identical MG191 SNP sequence types. The specimens from Patients 1 and 8 had the same numbers of STRs in the MG309 gene and the same amino acid change (Ser-80→Asn) in ParC. The specimens from Patients 3, 6, 10, 11, 12, 13, 16 and 17 had identical MG191 SNP sequence types. The specimens from Patients 3 and 12 had the same numbers of STRs in the MG309 gene and the same amino acid change (Ser-80→Asn) in ParC. The specimens from Patients 6 and 11 had the same numbers of STRs in the MG309 gene, but had different amino acid changes in ParC. The specimens from Patients 16 and 17 had the same numbers of STRs in the MG309 gene and the same mutation (A-2058→G) in the 23S rRNA gene. However, the specimen from Patient 16 had the amino acid change in ParC, whereas that from Patient 17 did not.

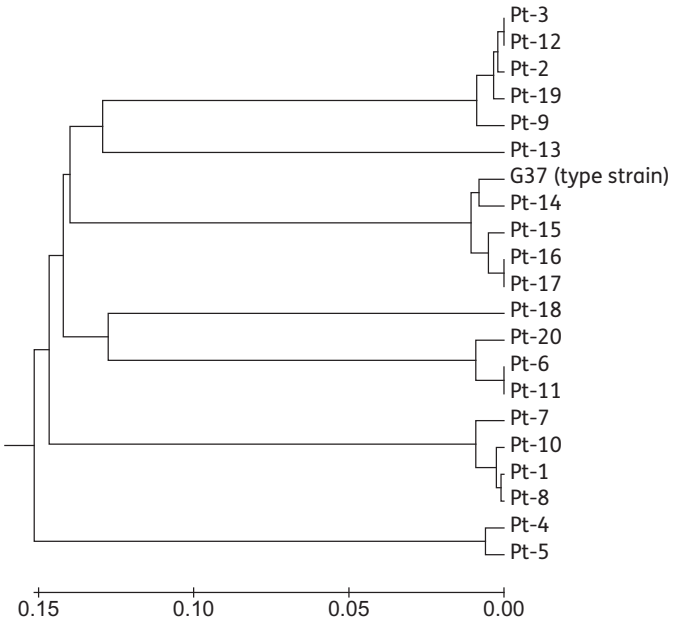

Figure 1. Dendrogram based on the combined sequences of the MG191 and MG309 genes determined from *M. genitalium* DNA specimens harbouring mutations in 23S rRNA and/or the *parC* genes and the type strain. The numbers on the right refer to the patients listed in Table 1.

Effects of antibiotic resistance-associated alterations in M. genitalium on microbiological outcomes of antibiotic treatments for M. genitalium-positive NGU

Of the 51 patients with NGU whose *M. genitalium* DNA specimens could be examined for the 23S rRNA, *gyrA* and *parC* genes, 31 were infected with *M. genitalium* and their DNA specimens had no macrolide and fluoroquinolone resistance-associated alterations. Of these 31 patients, 2 had mild symptoms and no pyuria in their FVU at their first visit, so they were not treated with antibiotics. Of the remaining 29 patients, 22, 6 and 1 were treated with a regimen of sitafloxacin at a dosage of 100 mg twice daily for 7 days, a regimen of 2 g of AZM-SR once daily for 1 day and a regimen of 500 mg of levofloxacin once daily for 7 days, respectively. Treatment failure occurred in two men, of whom one was treated with the AZM-SR regimen and the other was treated with the levofloxacin regimen. These two patients were then treated with sitafloxacin at a dosage of 100 mg twice daily for 7 days as the

second-line regimen and were microbiologically cured. Of the 20 patients infected with *M. genitalium* whose DNA specimens had macrolide and/or fluoroquinolone resistance-associated alterations, the AZM-SR regimen was prescribed in 7 NGU patients infected with *M. genitalium* harbouring only an amino acid change in ParC and in 2 men infected with mycoplasmas harbouring both a mutation in the 23S rRNA gene and an amino acid change in ParC (Table 1). In six NGU patients infected with *M. genitalium* harbouring only the amino acid change in ParC, the mycoplasma was eradicated with the AZM-SR regimen. However, in Patients 11 and 13 in Table 1 with NGU infected with *M. genitalium* harbouring the mutation in the 23S rRNA gene and the amino acid change Ser-80→Asn in ParC, the mycoplasma persisted after treatment with the AZM-SR regimen. These two patients were then treated with sitafloxacin at a dosage of 100 mg twice daily for 7 days as the second-line regimen and were microbiologically cured. The remaining 11 patients with NGU who were infected with *M. genitalium* harbouring a mutation in the 23S rRNA gene and/or an amino acid change in ParC were treated with the sitafloxacin regimen. All of them, including nine with NGU caused by the mycoplasma harbouring the single fluoroquinolone resistance-associated amino acid change in ParC, were microbiologically cured with the sitafloxacin regimen.

Discussion

In our previous studies, we found a macrolide resistance-associated mutation in the 23S RNA gene in only 1 (4%) of 25 *M. genitalium* DNA specimens collected from men with NGU between 2006 and 2008 and fluoroquinolone resistance-associated amino acid changes in ParC in 3 (10.7%) of 28 specimens.[16,21] In the present study, no macrolide resistance-associated mutations were found in *M. genitalium* in 2011 and 2012, but the macrolide resistance-associated mutations were observed in 5 (29.4%) of 17 specimens in 2013. The prevalence of fluoroquinolone resistance-associated amino acid changes at positions 80 and 84 in ParC seemed to increase steadily each year from 2011 to 2013. In 2013, 8 (47.1%) of 17 *M. genitalium* DNA specimens contained amino acid changes in ParC. For the first time, *M. genitalium* harbouring both the macrolide resistance-associated mutation in the 23S rRNA and the amino acid change in ParC was observed in 2013. In Australia and the UK, the prevalence of *M. genitalium* harbouring macrolide resistance-associated mutations exceeded 40% and was higher than that of the mycoplasmas harbouring fluoroquinolone resistance-associated amino acid changes in GyrA or ParC (4.5%–15.4%).[17,26] In this study, however, we found the prevalence of mycoplasmas with fluoroquinolone resistance-associated amino acid changes in ParC to be higher than that of mycoplasmas with macrolide resistance-associated mutations. In Japan, fluoroquinolone regimens have been recommended to treat chlamydial infections. In particular, levofloxacin regimens have often been prescribed to treat men with NGU; however, levofloxacin has only moderate activity against *M. genitalium*.[12] Takahashi *et al*.[27] reported that a regimen of 500 mg of levofloxacin once daily for 7 days eradicated *M. genitalium* in only three of five men with *M. genitalium*-positive NGU. In this study, we observed treatment failure with the levofloxacin regimen in one man infected with *M. genitalium*, whose DNA specimen had no fluoroquinolone resistance-associated amino acid changes in GyrA and ParC. In his *M. genitalium* DNA specimen collected after treatment, no mutations in the *gyrA* and *parC* genes were detected. In our previous study, however, we reported that the regimen of multiple low doses of levofloxacin selected *M. genitalium* harbouring the amino acid change Asp-80→Tyr in ParC after treatment.[28] Frequent use of fluoroquinolone regimens with moderate activity against *M. genitalium* in the treatment of urogenital infections could exert pressure to select mycoplasmas with fluoroquinolone resistance. Therefore, the high prevalence of fluoroquinolone resistance-associated amino acid changes in ParC observed in this study may be attributable to the frequent use of such fluoroquinolones in Japan.

Sixteen genotypes were observed in the 20 *M. genitalium* DNAs harbouring a macrolide resistance-associated mutation in the 23S rRNA gene and/or a fluoroquinolone resistance-associated amino acid change in ParC. Although some strains harbouring an identical fluoroquinolone resistance-associated amino acid change in ParC had identical genotypes, the multiclonal emergence of antibiotic-resistant *M. genitalium* may be occurring. The mycoplasma DNA from Patient 16, harbouring both a macrolide resistance-associated mutation in the 23S rRNA gene and a fluoroquinolone resistance-associated amino acid change in ParC, had a genotype identical to that from Patient 17, harbouring the same amino acid change in ParC. Strains with resistance to one antibiotic might evolve into strains with multidrug resistance by being exposed to another antibiotic in clinical practice.

In this study, treatment failure with the regimen of 2 g of AZM-SR once daily for 1 day was observed in one man infected with *M. genitalium* whose DNA specimen had no mutations in the 23S rRNA gene. In his *M. genitalium* DNA specimen collected after treatment, no mutations in the 23S rRNA gene were detected. Although Touati *et al*.[29] recently reported that an extended 5 day regimen of azithromycin selected a mycoplasma harbouring a macrolide resistance-associated mutation in the 23S rRNA gene in clinical practice, extended regimens have been expected to decrease the risk of macrolide resistance selection during azithromycin treatments for *M. genitalium* infections.[2,30] The AZM-SR regimen could also contribute to the prevention of the selection of macrolide-resistant *M. genitalium*. However, this regimen failed to eradicate the mycoplasma harbouring the macrolide resistance-associated mutation in the 23S rRNA gene in two men. Mycoplasmas harbouring a single mutation in the 23S rRNA gene would be resistant to the AZM-SR regimen.

The sitafloxacin regimen succeeded in eradicating *M. genitalium* harbouring fluoroquinolone resistance-associated amino acid changes in ParC in all 11 treated men, including 2 after azithromycin treatment failure. This regimen could overcome the fluoroquinolone resistance that is conferred on *M. genitalium* by single amino acid changes in ParC. However, the acquisition of a single amino acid change in GyrA or ParC might be the first step in the development of clinically significant resistance to fluoroquinolones. Subsequently, the accumulation of amino acid changes in GyrA and ParC could be induced by serial exposure to fluoroquinolones of strains with a single amino acid change in GyrA or ParC and could bring about stepwise increases in the level of fluoroquinolone resistance.[31] The frequent use of the sitafloxacin regimen might select mutants with high-level resistance to fluoroquinolones, including sitafloxacin. Therefore, the remarkable increase in *M. genitalium* strains harbouring single amino acid changes in ParC would be a matter of great concern for the fluoroquinolone treatment of *M. genitalium* infections.

In conclusion, macrolide- or fluoroquinolone-resistant *M. genitalium* appears to be increasing, and the increase in fluoroquinolone-resistant mycoplasmas is especially remarkable in Japan. Mycoplasmas harbouring the single mutation in the 23S rRNA gene would be resistant to the AZM-SR regimen, whereas those harbouring the single amino acid change in ParC would still be susceptible to the sitafloxacin regimen. We are fully aware of the limitations of this study, including the small number of specimens and the lack of analysis of antimicrobial susceptibilities of isolated strains harbouring macrolide and/or fluoroquinolone resistance-associated alterations, which were found in this study. Additionally, further studies are needed to examine the microbiological efficacy of the AZM-SR and sitafloxacin regimens against *M. genitalium* infections in a large number of subjects and confirm that these regimens do not bring about the selection of antibiotic resistance in *M. genitalium* or increase the levels of its acquired antibiotic resistance. However, our present findings that macrolide- or fluoroquinolone-resistant *M. genitalium* appears to be increasing and that the increase in fluoroquinolone-resistant mycoplasmas is especially remarkable in Japan would suggest that, instead of the 1 g single-dose regimen of azithromycin, the AZM-SR regimen might become the first-line treatment for *M. genitalium* infections and that the sitafloxacin regimen might become the second- or successive-line treatment in Japan. Before strains with clinically significant higher-level resistance to fluoroquinolones, including moxifloxacin and sitafloxacin, emerge, however, promising new antibiotic regimens for *M. genitalium* infections must be developed and be available in clinical settings.

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Transparency declarations

None to declare.

Author contributions

Study concept and design: M. K., S. I. and T. D. Acquisition of data: M. K., S. I., K. H. and M. T. Analysis and interpretation of data: M. K., S. I., M. Y., T. T., T. E. and T. D. Drafting of manuscript: M. K. and T. D. Critical revision of the manuscript for important intellectual content: S. I., M. Y., T. T. and T. E. Obtaining funding: T. D. Supervision: T. D.

References

1 Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: from chrysalis to multicolored butterfly. *Clin Microbiol Rev* 2011; **24**: 498–514.

2 Deguchi T, Ito S, Hagiwara N *et al*. Antimicrobial chemotherapy of *Mycoplasma genitalium*-positive non-gonococcal urethritis. *Expert Rev Anti Infect Ther* 2012; **10**: 791–803.

3 Jensen JS, Bradshaw CS, Tabrizi SN *et al*. Azithromycin treatment failure in *Mycoplasma genitalium*-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. *Clin Infect Dis* 2008; **47**: 1546–53.

4 Bradshaw CS, Jensen JS, Tabrizi SN *et al*. Azithromycin failure in *Mycoplasma genitalium* urethritis. *Emerg Infect Dis* 2006; **12**: 1149–52.

5 Vester B, Douthwaite S. Macrolide resistance conferred by base substitutions in 23S rRNA. *Antimicrob Agents Chemother* 2001; **45**: 1–12.

6 Fraser CM, Gocayne JD, White O *et al*. The minimal gene complement of *Mycoplasma genitalium*. *Science* 1995; **270**: 397–403.

7 Ito S, Shimada Y, Yamaguchi Y *et al*. Selection of *Mycoplasma genitalium* strains harbouring macrolide resistance-associated 23S rRNA mutations by treatment with a single 1 g dose of azithromycin. *Sex Transm Infect* 2011; **87**: 412–4.

8 Yew HS, Anderson T, Coughlan E *et al*. Induced macrolide resistance in *Mycoplasma genitalium* isolates from patients with recurrent nongonococcal urethritis. *J Clin Microbiol* 2011; **49**: 1695–6.

9 Liu P, Allaudeen H, Chandra R *et al*. Comparative pharmacokinetics of azithromycin in serum and white blood cells of healthy subjects receiving a single-dose extended-release regimen versus a 3-day immediate-release regimen. *Antimicrob Agents Chemother* 2007; **51**: 103–9.

10 Bradshaw CS, Chen MY, Fairley CK. Persistence of *Mycoplasma genitalium* following azithromycin therapy. *PLoS One* 2008; **3**: e3618.

11 Jernberg E, Moghaddam A, Moi H. Azithromycin and moxifloxacin for microbiological cure of *Mycoplasma genitalium* infection: an open study. *Int J STD AIDS* 2008; **19**: 676–9.

12 Hamasuna R, Jensen JS, Osada Y. Antimicrobial susceptibilities of *Mycoplasma genitalium* strains examined by broth dilution and quantitative PCR. *Antimicrob Agents Chemother* 2009; **53**: 4938–9.

13 Ito S, Yasuda M, Seike K *et al*. Clinical and microbiological outcomes in treatment of men with non-gonococcal urethritis with a 100-mg twice-daily dose regimen of sitafloxacin. *J Infect Chemother* 2012; **18**: 414–8.

14 Takahashi S, Hamasuna R, Yasuda M *et al*. Clinical efficacy of sitafloxacin 100 mg twice daily for 7 days for patients with non-gonococcal urethritis. *J Infect Chemother* 2013; **19**: 941–5.

15 Waites KB, Lysnyansky I, Bebear C. Emerging antimicrobial resistance in mycoplasmas of humans and animals. In: Browning GF, Citti C, eds. *Mollicutes: Molecular Biology and Pathogenesis*. Norwich: Caister Academic Press, 2014; 289–322.

16 Shimada Y, Deguchi T, Nakane K *et al*. Emergence of clinical strains of *Mycoplasma genitalium* harbouring alterations in ParC associated with fluoroquinolone resistance. *Int J Antimicrob Agents* 2010; **36**: 255–8.

17 Tagg KA, Jeoffreys NJ, Couldwell DL *et al*. Fluoroquinolone and macrolide resistance-associated mutations in *Mycoplasma genitalium*. *J Clin Microbiol* 2013; **51**: 2245–9.

18 Yoshida H, Bogaki M, Nakamura M *et al*. Quinolone resistance-determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. *Antimicrob Agents Chemother* 1990; **34**: 1271–2.

19 Couldwell DL, Tagg KA, Jeoffreys NJ *et al*. Failure of moxifloxacin treatment in *Mycoplasma genitalium* infections due to macrolide and fluoroquinolone resistance. *Int J STD AIDS* 2013; **24**: 822–8.

20 Yoshida T, Maeda S, Deguchi T *et al*. Rapid detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum*, and *Ureaplasma urealyticum* organisms in genitourinary samples by PCR-microtiter plate hybridization assay. *J Clin Microbiol* 2003; **41**: 1850–5.

21 Shimada Y, Deguchi T, Nakane K *et al*. Macrolide resistance-associated 23S rRNA mutation in *Mycoplasma genitalium*, Japan. *Emerg Infect Dis* 2011; **17**: 1148–50.

22 Hjorth SV, Björnelius E, Lidbrink P *et al*. Sequence-based typing of *Mycoplasma genitalium* reveals sexual transmission. *J Clin Microbiol* 2006; **44**: 2078–83.

23 Ma L, Taylor S, Jensen JS *et al*. Short tandem repeat sequences in the *Mycoplasma genitalium* genome and their use in a multilocus genotyping system. *BMC Microbiol* 2008; **8**: 130.

24 Musatovova O, Baseman JB. Analysis identifying common and distinct sequences among Texas clinical strains of *Mycoplasma genitalium*. *J Clin Microbiol* 2009; **47**: 1469–75.

25 Cazanave C, Charron A, Renaudin H *et al*. Method comparison for molecular typing of French and Tunisian *Mycoplasma genitalium*-positive specimens. *J Med Microbiol* 2012; **61** Pt 4: 500–6.

26 Pond MJ, Nori AV, Witney AA *et al*. High prevalence of antibiotic-resistant *Mycoplasma genitalium* in nongonococcal urethritis: the need for routine testing and the inadequacy of current treatment options. *Clin Infect Dis* 2014; **58**: 631–7.

27 Takahashi S, Ichihara K, Hashimoto J *et al*. Clinical efficacy of levofloxacin 500 mg once daily for 7 days for patients with non-gonococcal urethritis. *J Infect Chemother* 2011; **17**: 392–6.

28 Deguchi T, Maeda S, Tamaki M *et al*. Analysis of the *gyrA* and *parC* genes of *Mycoplasma genitalium* detected in first-pass urine of men with non-gonococcal urethritis before and after fluoroquinolone treatment. *J Antimicrob Chemother* 2001; **48**: 742–4.

29 Touati A, Peuchant O, Jensen JS *et al*. Direct detection of macrolide resistance in *Mycoplasma genitalium* isolates from clinical specimens from France by use of real-time PCR and melting curve analysis. *J Clin Microbiol* 2014; **52**: 1549–55.

30 Anagrius C, Loré B, Jensen JS. Treatment of *Mycoplasma genitalium*. Observations from a Swedish STD clinic. *PLoS One* 2013; **8**: e61481.

31 Fàbrega A, Madurga S, Giralt E *et al*. Mechanism of action of and resistance to quinolones. *Microb Biotechnol* 2009; **2**: 40–61.