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# Efficacy of Doxycycline-Sitafloxacin Sequential Therapy for Urogenital *Mycoplasma genitalium* Infection in Nanjing, China

*Mengjin Yuan, MD, Wenjing Le, MS, Yuanyuan Zhao, MD, PhD, Lu Gan, MD, PhD, Sai Li, MD, PhD, and Xiaohong Su, MD, PhD*

**Background:** The aim of this study was to evaluate the efficacy of doxycycline-sitafloxacin sequential therapy in the treatment of *Mycoplasma genitalium* (*Mg*) urogenital infections in Nanjing, China.

**Methods:** Potential subjects were tested initially for *Mg* infection by nucleic acid amplification testing and again at least 21 days after completion of doxycycline (100 mg twice daily for 7 days)–sitafloxacin (100 mg twice daily for 7 days) sequential therapy. The presence of macrolide and quinolone resistance–associated mutations in 23S rRNA, *parC*, *gyrA*, and *gyrB* genes in *Mg* was examined at baseline and upon retesting of specimens from subjects that did not clear *Mg*.

**Results:** A total of 218 patients were screened for *Mg*, of whom 65 were positive for *Mg*; 63 *Mg*-infected patients were enrolled. Twenty-two (35%) *Mg*-infected subjects (16 heterosexual men, 5 women, and 1 man who had sex with men [MSM]) were successfully evaluated with a test of cure; 20 (91%) cleared *Mg* infection. In pretreatment specimens, mutations in 23S rRNA, *parC* (G248T [S83I]), *gyrA* (G277T [G93C]), and *gyrB* genes were present in 100% (19 of 19), 61.1% (11 of 18), 6.7% (1 of 15), and 7.1% (1 of 14), respectively. *Mg* clearance rates were 4 of 4 in infected subjects that possessed both wild-type *parC* and *gyrA* genes, and 9 of 10 when a *parC* G248T mutation and an otherwise wild-type *gyrA* gene were identified. Two subjects (9%) reported mild adverse events.

**Conclusions:** Doxycycline-sitafloxacin sequential therapy was well tolerated and effective against most urogenital *Mg* infections in Nanjing and may provide an option for treatment.

*Mycoplasma genitalium* (*Mg*) is a sexually transmitted bacterial pathogen that causes nongonococcocal urethritis in men.[1] *Mg* is also associated with epididymitis, and proctitis in men and cervicitis, pelvic inflammatory disease, premature delivery, and miscarriage in women. Asymptomatic *Mg* infection is common in both men and women.[2] *Mg* infection can also increase the risk of transmission or acquisition of HIV infection.[3,4]

Antimicrobial resistance in *Mg* to currently recommended therapies continues to rise worldwide.[5–7] First-line therapy entails doxycycline followed by azithromycin or a fluoroquinolone (e.g., moxifloxacin).[8–10] However, a high prevalence of macrolide and fluoroquinolone resistance has been reported in the Asia-Pacific region; macrolide resistance was greater than 50%, and fluoroquinolone resistance was 10% to 27%, in Australia, New Zealand, and Japan.[5,11,12] A meta-analysis that included 20 studies, published from 2013 to 2023, reported that the prevalence of genetic mutations in *Mg* associated with resistance to macrolides and fluoroquinolones was 43.5% (95% confidence interval [CI], 33.1%–54.5%) and 18.6% (95% CI, 13.2%–25.5%), respectively; the prevalence of multidrug resistance was 17.4% (95% CI, 9.2%–30.5%).[13]

In China, antimicrobial resistance in *Mg* is even greater. The prevalences of macrolide and quinolone resistance–associated mutations in 23S rRNA and *parC* genes were 88.9% (303 of 341) and 89.5% (308 of 344), respectively, in men with symptomatic urethritis in our Nanjing sexually transmitted disease (STD) clinic; the prevalence of these mutations together was 88% (270 of 308).[14] Mutations in 23S rRNA and *parC* genes were reported in 66.4% (101 of 152) and 77.7% (108 of 139) of urethral specimen in Guangzhou STD clinics,[15] and 83% (73 of 88) and 79.8% (71 of 89) of urethral and rectal specimens in Chinese men who have sex with men (MSM).[16]

Effective treatment of *Mg* is challenging because of increasing antibiotic resistance and failing efficacy of recommended first-line therapies. Azithromycin failure rates in *Mg*-positive nongonococcocal urethritis were reported to be 28% (95% CI,

From the Sexually Transmitted Disease (STD) Clinic, Hospital for Skin Diseases, Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College, Nanjing, China

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Correspondence: Xiaohong Su, MD, PhD, Sexually Transmitted Disease (STD) Clinic, Hospital for Skin Diseases, Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 12 Jiangwangmiao Road, Nanjing 210042, Jiangsu Province, China. E-mail: suxh@ncstdlc.org. Sai Li, MD, PhD, Sexually Transmitted Disease (STD) Clinic, Hospital for Skin Diseases, Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 12 Jiangwangmiao Road, Nanjing 210042, Jiangsu Province, China. E-mail: wslisai@163.com.

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15%–45%) in Melbourne, Australia,[17] and 60.5% (95% CI, 43.4–76.0%) in Seattle, Washington.[18] Moreover, fluoroquinolone resistance is also increasing, including treatment failures when moxifloxacin was used.[19,20] To improve management of *Mg* infection in the face of rising antibiotic resistance, resistance-guided therapy (RGT) has been proposed[21,22]; currently, RGT is recommended in the United States, British, and Australian sexually transmitted infection (STI) treatment guidelines.[8–10] Resistance-guided therapy is based on results of testing for macrolide resistance; therapy with doxycycline is followed by azithromycin for macrolide-sensitive *Mg* infections—sequential therapy; doxycycline is followed by moxifloxacin or sitafloxacin for macrolide-resistant infections.[21,22] Treatment failure rates were reduced from 27% to 3% after implementation of RGT in a UK sexual health center.[23] Unfortunately, molecular testing for macrolide resistance in *Mg* is not readily available in China. Previously, we investigated the efficacy of doxycycline (7 days) followed by moxifloxacin (7 days)—sequential therapy in the treatment of *Mg* infection in the Nanjing STD clinic and observed a microbiological clearance rate of 66.7% (12 of 18),[24] lower than reported in Australia (92.0% [95% CI, 88.1%–94.6%]).[22] In a retrospective study conducted in fertility and STD clinics in Guangzhou, China, the overall treatment failure rate was 28.29% (71 of 251) in patients with *Mg* infection; failure rates of doxycycline, azithromycin, and moxifloxacin were 27.9% (29 of 104), 44% (22 of 50), and 50% (2 of 4), respectively.[25]

Sitafloxacin is a fourth-generation fluoroquinolone with higher in vitro potency against *Mg* than moxifloxacin.[26] Although microbiological cure rates of *Mg* are reported similar in studies of doxycycline-moxifloxacin versus doxycycline-sitafloxacin sequential therapy[21,22]; nonetheless, doxycycline-sitafloxacin was more efficacious against *Mg* infections that carried the ParC S83I mutation.[20] Combination therapy with doxycycline and sitafloxacin successfully cleared 11 of 12 cases of highly resistant *Mg* infection, which had first failed sequential-based therapies with doxycycline and moxifloxacin (or moxifloxacin alone) and then also failed pristinamycin.[27]

Access to sitafloxacin is limited in many countries. It is available, however, in the Asia-Pacific region but has not been used to treat *Mg* infection in China. Because of high levels of fluoroquinolone resistance combined with potentially lower efficacy of moxifloxacin in China,[14–16,24,25] more effective treatment options need to be identified. The primary aim of this study was to evaluate *Mg* clearance in subjects treated with doxycycline-sitafloxacin sequential therapy. An additional aim was to evaluate tolerability and adherence to doxycycline-sitafloxacin sequential therapy. Association between treatment outcomes and macrolide and quinolone resistance–associated mutations (MRAMs and QRAMs) in 23S rRNA, *parC*, *gyrA*, and *gyrB* genes was also investigated.

## SUBJECTS AND METHODS

### Study Population and Data Collection

We conducted an open-label, single-center prospective cohort study at the STD Clinic, Hospital for Skin Diseases, Institute of Dermatology, Chinese Academy of Medical Sciences, in Nanjing, China, from April 2022 to April 2023. Most patients who presented with urethritis, cervicitis, and/or self-reported sexual contact with a *Mg*-infected partner were tested for *Mg* by polymerase chain reaction (PCR; Rendu Biotecnology). In men, urethritis was defined as ≥2 polymorphonuclear leukocytes per high-power field plus urethral symptoms or visible discharge. Cervicitis was defined as (1) a purulent or mucopurulent endocervical exudate visible in the endocervical canal on speculum examination or on an endocervical swab specimen, and (2) sustained endocervical bleeding, easily induced by gentle passage of a cotton swab through the cervical os. Participants were also tested for *Neisseria gonorrhoeae* (*Ng*) by Gram stain, culture (modified Thayer-Martin medium; Zhuhai DL Biotech Co. Ltd.), and/or PCR (DAAN Gene Co. Ltd.); *Chlamydia trachomatis* (*Ct*) by PCR (DAAN Gene Co. Ltd.); and *Ureaplasma urealyticum* (*Uu*) and *Mycoplasma hominis* (*Mh*) by culture (Mycoplasma IST2; bioMerieux). Patients with active syphilis (microscopic darkfield examination and/or rapid plasma reagin positive and *Treponema pallidum* passive particle agglutination assay) and *Ng* infection detected by Gram stain (males), culture, or PCR were excluded from the study to avoid excessive use of antimicrobials—trial therapies plus first-line therapies for syphilis and gonorrhea. In eligible patients who then signed informed consent, doxycycline was prescribed presumptively (100 mg twice daily for 7 days). *Mg*-infected subjects were seen again in the clinic after 1 week and given sitafloxacin (100 mg twice daily for 7 days). Enrolled subjects were scheduled to return for a test of clearance (TOC) at least 21 days after completion of the sequential regimen. Recorded demographic characteristics included gender and sexual orientation, marital status, and number of recent (3 months) sexual partners. Also tabulated were a history of *Mg* infection, use of antibiotics in the 30 days before enrollment, and coinfection with other STI-related microbes. A study-specific recording instrument (including a questionnaire) was designed to collect data at the first and TOC visit. Symptoms and clinical signs were recorded at the first visit. At the TOC visit, residual symptoms/clinical signs, adherence to the antimicrobial regimen, adverse reactions, sexual contacts, and treatment of sex partners were identified and recorded. Clinical and laboratory data were also recorded at the TOC visit.

### Efficacy and Adverse Reactions

Microbiological clearance was defined as a negative result for *Mg* by PCR at the TOC visit (at least 21 days after completion of treatment); clinical cure was defined as resolution of all symptoms and clinical signs. Adverse reactions were also recorded.

### Determination of Antimicrobial Resistance

First voided urine from men and cervical swab specimens from women were collected before and after sequential therapy and stored at −80°C. DNA was extracted from *Mg*-positive samples using the Rapid Bacterial Genomic DNA Isolation Kit (DNA Quick Extract Kit; Epicentre). Macrolide resistance–associated mutations (MRAMs) in region V of the 23S rRNA gene (nucleotides 1992–2138) and quinolone resistance–associated mutations (QRAMs) in the quinolone resistance–determining regions (QRDRs) of *parC* (nucleotides 164–477), *gyrA* (nucleotides 172–402), and *gyrB* genes (nucleotides 1256–1480) were detected by nested PCR and confirmed by DNA sequencing. Primers used for amplification of these genes have been described previously.[14,28] Gene amplification products were sequenced in both directions (GENEWIZ From Azenta Life Science Co Ltd), gene sequences aligned against corresponding sequences in *Mg* G37 (GenBank accession number NC_00908.2; https://www.ncbi.nlm.nih.gov/genome/?term=mycoplasma+genitalium), and mutations identified.

### Statistical Analysis

Statistical analyses were conducted using SPSS Statistics version 24. Treatment efficacy was calculated with 95% CIs of proportions calculated by exact methods. The $\chi^2$ test and Fisher exact test were used to compare categorical variables.

## RESULTS

### Enrollment

From April 2022 to April 2023, 244 patients with urethritis, cervicitis, and/or self-reported sexual contact with an *Mg*-infected partner were treated at the Nanjing STD clinic. A total of 218 patients were identified as potential subjects and screened for *Mg* infection; 26 patients were not screened (excluded) for reasons indicated in Figure 1, including 8 who declined participation in the trial, a priori. One hundred fifty-five additional patients were excluded for reasons indicated in Figure 1 (including 153 who had a negative *Mg* PCR). Sixty-three were *Mg* infected and were enrolled, and sequential therapy was initiated as part of the study; 27 were excluded after/during the doxycycline phase (25 declined further participation; 2 were lost to follow-up; Fig. 1). The 36 subjects who remained entered the sitafloxacin phase of the study; 14 were excluded after/during the sitafloxacin phase (12 did not return for TOC; 1 was lost to follow-up and 1 discontinued therapy because of an adverse event; Fig. 1). Twenty-two subjects (35% [22 of 63]) completed the study; 20 of 22 (90.9%) cleared *Mg* infection.

### Characteristics of 22 Subjects Who Completed the Trial

There were 16 heterosexual men (72.7%) and 5 women (22.7%), including 2 pairs who were sex partners. One man had sex with men (MSM). Participants' age ranged from 19 to 58 years; median age was 30 (interquartile range, 28–43) years. Twenty-one of 22 subjects (95.5%) were infected with *Mg* for the first time; one man had been infected with *Mg* in the past and had received doxycycline-moxifloxacin sequential treatment at that time. Nineteen of 22 (86.4%) were symptomatic or had clinical signs at the time of enrollment: urethritis in men (n = 16) and cervicitis in women (n = 3). Demographic characteristics of study participants are presented in Table 1.

### Treatment Outcome, and *Mg* Clearance and Resolution of Symptoms and Clinical Signs

Twenty of 22 patients (90.9% [95% CI, 72.2%–97.5%]) subjects cleared *Mg* infection following sequential therapy with doxycycline-sitafloxacin. The median time to TOC was 23 (range, 20–86) days. Prior symptoms and clinical signs resolved completely

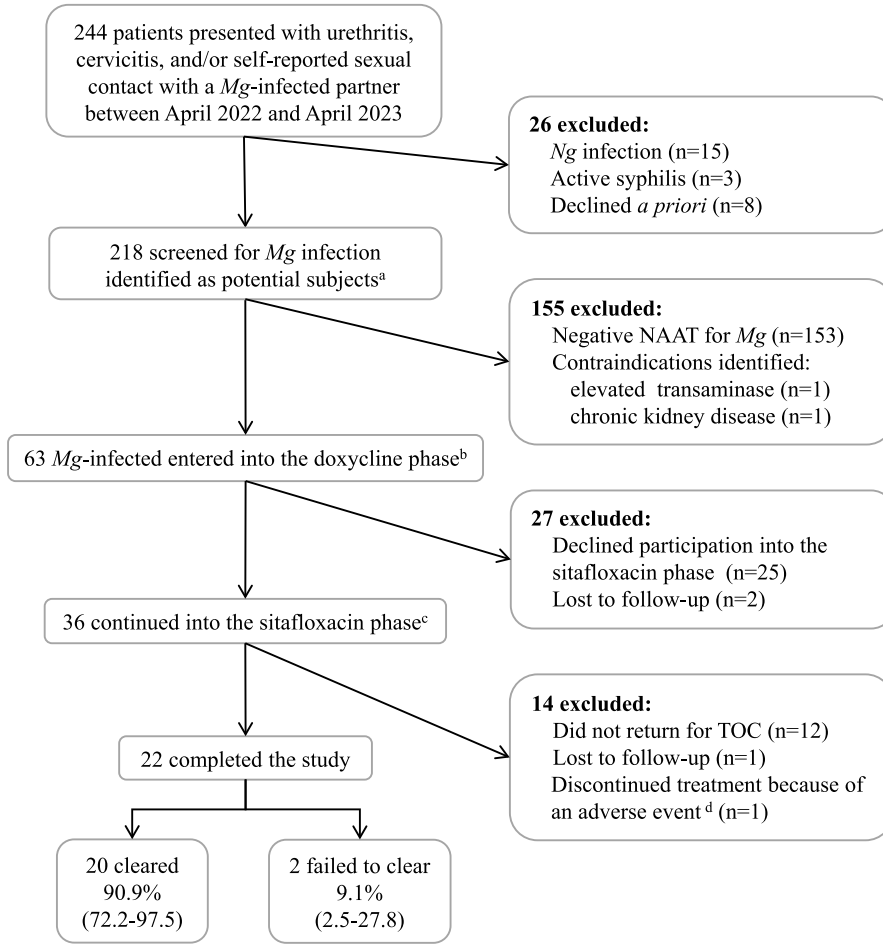


**Figure 1.** Flowchart showing the selection of *Mg*-positive participants for the doxycycline-sitafloxacin sequential therapy study. A, There were 190 men and 28 women (186 cases of urethritis in men, 20 cases of cervicitis, and 12 cases otherwise exposed to a previously *Mg*-infected partner). B, There were 55 men and 8 women, including 49 cases of male urethritis, 6 cases of cervicitis, and 8 asymptomatic infections. C, There were 30 men (including 1 MSM) and 6 women (28 cases of urethritis in men, 4 cases of cervicitis, and 4 asymptomatic infections). D, Extremity weakness appeared on the second day of sitafloxacin stage and resolved completely within 1 week of discontinuation.

**TABLE 1.** Characteristics of Study Participants (n = 22)

| Characteristic | No. (%) |
|---|---|
| Demographic | 30 (28–43) |
| Median age (IQR), y | |
| Gender/sexual orientation | |
| Female | 5 (22.7) |
| Heterosexual male | 16 (72.7) |
| MSM | 1 (4.6) |
| Marital status | |
| Married | 11 (50.0) |
| Unmarried | 11 (50.0) |
| No. sexual partners in past 3 mo | |
| 1 | 17 (77.3) |
| ≥2 | 4 (18.2) |
| NK | 1 (4.5) |
| Antibiotic use and microbiology | |
| Antibiotic use in the prior 30 d | |
| No | 11 (50.0) |
| Doxycycline | 3 (13.6) |
| Minocycline | 3 (13.6) |
| Roxithromycin | 2 (9.1) |
| Azithromycin | 1 (4.5) |
| Amoxicillin | 1 (4.5) |
| Levofloxacin | 1 (4.5) |
| Prior *M. genitalium* infection | |
| No | 21 (95.5) |
| Yes | 1 (4.5) |
| Coinfection with other pathogens | |
| No coinfection | 16 (72.7) |
| *Ct* | 2 (9.1) |
| *Uu* | 4 (18.2) |
| *Mh* | 3 (13.6) |

IQR indicates interquartile range; MSM, men who have sex with men; NK, not known.

in subjects who cleared *Mg* infection. Two men who failed therapy had persistent but mild symptoms of urethritis; the man with prior *Mg* infection, who earlier had failed doxycycline-moxifloxacin sequential therapy, cleared *Mg* infection after doxycycline-sitafloxacin; and prior symptoms and clinical signs resolved completely. Chlamydia (*Ct*) and Ureaplasma (*Uu*) test results of 6 coinfected subjects were negative after treatment. All subjects denied possibility of reinfection during treatment and follow-up.

## Adherence and Adverse Events

A majority of subjects (90.9% [20 of 22]) reported taking all doses of prescribed antibiotics. Two subjects reported adverse events; one during the doxycycline phase—nausea, and the other during the sitafloxacin phase—mild bilateral Achilles tendon pain but completed the trial, nonetheless. One subject dropped out of the trial because of an adverse event—extremity weakness, which came on the second day of sitafloxacin intake. Upon a follow-up telephone call to the subject, assessment of muscle tension and measurement serum electrolyte concentrations (both done at another hospital) were reported as normal. Sitafloxacin was stopped immediately, and symptoms were resolved within a week.

## Mutations in 23S rRNA, *parC*, *gyrA*, and *gyrB* Genes in Pretreatment Specimens

Twenty-two pretreatment *Mg*-positive samples were analyzed. Sequencing of the 23S rRNA gene was successful in 19 (86.4%); mutations in the 23S rRNA gene were detected in all 19. An A2059G mutation was present in 13 *Mg* sequences, A2058T in 3, A2058G in 2, and A2059C in 1 (Table 2). The *parC* gene was successfully amplified and sequenced from 18 samples; 6 were wild type (WT) and 12 possessed a mutation(s). Eleven of 18 (61.1%) possessed a single G248T missense mutation that resulted in serine (S) substitution at position 83 (S83I) in ParC (G248T [S83I]); another possessed a (silent) single T210C mutation (Table 2). The *gyrA* gene was successfully amplified and sequenced from 15 samples; 13 were WT and 2 possessed mutations: a single G277T missense mutation (1 of 15 [6.7%]) that resulted in glycine (G) substitution at position 93 (G93C) in GyrA (G277T [G93C]) and another that possessed a (silent) single G240A mutation (Table 2). The *gyrB* gene was successfully amplified and sequenced from 14 samples; one possessed a single C1384T missense mutation (1 of 14 [7.1%]) that resulted in proline (P) substitution at position 462 (C1384T [P462S]) in GyrB (Table 2). Of the pretreatment samples successfully tested for MRAMs and QRAMs, 11 of 14 (78.6%) possessed both MRAM and QRAM (*parC* [S83I]) mutations, and 1 possessed triple MRAM and QRAM (*parC* [S83I] and *gyrA* [G93C]) mutations.

**TABLE 2.** Frequency of Mutations Identified in the 23S Ribosomal RNA, *parC*, *gyrA*, and *gyrB* Genes in Pretreatment Specimens

| Gene | Mutation | Amino Acid Change | No. Samples (n) | Frequency, % |
|---|---|---|---|---|
| 23S rRNA (n = 19) | A2058T | Silent mutation | 3 | 15.8 |
| | A2058G | Silent mutation | 2 | 10.5 |
| | A2059G | Silent mutation | 13 | 68.4 |
| | A2059C | Silent mutation | 1 | 5.3 |
| *parC* (n = 18) | T210C | Silent mutation | 1 | 5.6 |
| | G248T | S83I | 11 | 61.1 |
| | WT | — | 6 | 33.3 |
| *gyrA* (n = 15) | G240A | Silent mutation | 1 | 6.7 |
| | G277T | G93C | 1 | 6.7 |
| | WT | — | 13 | 86.6 |
| *gyrB* (n = 14) | C1384T | P462S | 1 | 7.1 |
| | WT | — | 13 | 92.9 |
| *parC*·*gyrA*·*gyrB* (n = 14) | G248T·WT·WT | S83I·WT·WT | 9 | 64.3 |
| | G248T·G277T·WT | S83I·G93C·WT | 1 | 7.1 |
| | G248T·WT·C1384T | S83I·WT·P462S | 1 | 7.1 |
| | WT·WT·WT WT·G240A·WT | WT·WT·WT | 3 | 21.4 |

rRNA indicates ribosomal RNA; WT, wild type.

**TABLE 3.** Microbiological Clearance Rate of *M. genitalium* Infections According to the Presence of Fluoroquinolone-Resistance Mutations

| Gene | Mutation | Amino Acid Change | Microbiological Clearance Rate, n (%) |
|---|---|---|---|
| *parC* | G248T | S83I | 9/11 (81.8) |
| | W1 | WT | 7/7 (100) |
| *gyrA* | G277T | G93C | 0/1 (0) |
| | W2 | WT | 13/14 (92.9) |
| *parC*·*gyrA* | W1, W2 | WT | 4/4 (100) |
| | G248T·W2 | S83I·WT | 9/10 (90) |
| | G248T·G277T | S83I·G93C | 0/1 (0) |

W1, mutations in which no amino acid changes in WT ParC were present, including no T210C in *parC*; W2 indicates mutations in which no amino acid change in WT GyrA were present including no G240T in *gyrA*; WT, wild type.

### Treatment Outcomes as a Function of Antimicrobial Resistant Genes

The clearance rate of *Mg* was 81.8% (9 of 11) in subjects in which *Mg* sequences in their samples possessed the *parC* S83I mutation (Table 3). *Mg* clearance was 100% (4 of 4) in samples from infected subjects who possessed both WT *parC* and WT *gyrA* genes (*parC*·*gyrA*) and 90% (9 of 10) when a *parC* mutation (G248T [S83I]) and an otherwise WT *gyrA* gene were identified. There were 3 subjects whose *Mg*-infected specimens had *gyrA* mutations in pretreatment samples; one failed sequential therapy.

One subject who earlier had failed to clear *Mg* with doxycycline-moxifloxacin sequential therapy cleared *Mg* with doxycycline-sitafloxacin. The *parC* G248T (S83I) mutation and an otherwise WT *gyrA* gene were identified in his pretreatment samples. The 2 subjects who failed sequential therapy possessed the same mutations in pretreatment and posttreatment samples; one possessed a G248T (S83I) mutation in the *parC* gene, and the other possessed a G248T (S83I) mutation in *parC* plus a G277T (G93C) mutation in *gyrA*.

## DISCUSSION

Our study reports the efficacy of sequential therapy with doxycycline-sitafloxacin for treatment of urogenital *Mg* infection in Nanjing, China, where rates of macrolide and fluoroquinolone resistance–associated mutations are high.[14]

In light of the global increase in macrolide-resistant *Mg*, widely recommended guidelines propose sequential therapy with doxycycline and moxifloxacin for the treatment of macrolide-resistant *Mg* infections.[6,8,10,23] Doxycycline-moxifloxacin sequential therapy results in clearance of *Mg* in more than 85% of subjects infected with *Mg* that possess macrolide resistance–associated mutations (MRAMs).[21,22,29] The efficacy of moxifloxacin in macrolide-resistant *Mg* infections depends almost entirely on the quinolone resistance–associated mutations (QRAMs), especially the *parC* S83I mutation with or without specific *gyrA* mutations.[20] However, sitafloxacin, a newer-generation fluoroquinolone, may have greater activity against *Mg* that possess QRAMs. Quinolone-selected *Mg* mutants of ATCC 33530 that possessed 4 to 16 times higher mean inhibitory concentrations (MICs) against ciprofloxacin than that of the parent strain, ATCC 33530 itself, possessed MICs against sitafloxacin (0.125–0.25 mg/L) that were similar to that in the parent strain (0.125 mg/L).[30] *Mg* that possessed ParC S83I mutations also had lower MICs to sitafloxacin than to moxifloxacin.[31s] Sitafloxacin has been shown to be clinically effective against *Mg* that possessed MRAMs and highly resistant *Mg* that possessed QRAMs.[20,21,27]

Fluoroquinolone resistance and treatment failures have been associated with certain QRAMs (e.g., mutated *parC* and *gyrA* genes), although correlation of treatment outcomes with specific QRAMs has not been determined with certainty. The *parC* G248T mutation, which results in an amino acid change at S83I in ParC, is the most common mutation associated with both moxifloxacin and sitafloxacin treatment failures.[20,32s,33s] Macrolide-resistant *Mg* infections that lacked the ParC S83I mutation had a 96.4% (95% CI, 93.7%–98.2%) probability of clearance when moxifloxacin was used for treatment.[32s] However, when the ParC S83I mutation is present, treatment failures with moxifloxacin rise to 59% and 63%.[29,32s,33s] Concurrent GyrA mutations in *Mg*, particularly M95I combined with D99N, result in greater numbers of treatment failures with either moxifloxacin or sitafloxacin than similar treatment of *Mg* that possess the ParC S83I mutation alone.[20,33s]

In China, a high level of fluoroquinolone resistance mutations is present in *Mg* infections.[14,15] ParC S83I mutations have been reported in 71% to 84% of *Mg*-positive samples in China and Japan,[14,15,34s] which is higher than the presence of this mutation in *Mg* in Australia and the United States.[35s,36s] In China, the prevalence of *Mg gyrA* mutation is even higher compared with other countries.[11,14,20,36s–38s] Here, we showed that the rate of MRAMs and the quinolone resistance–associated ParC (S83I) mutation was high (100% [19 of 19] and 61% [11 of 18], respectively). Clearance of *Mg* was complete in subjects with *Mg* sequences that possessed both WT ParC and WT GyrA and 90% (9 of 10) for *Mg* that possessed the ParC S83I mutation and WT GyrA, similar to a study in Japan that examined sitafloxacin monotherapy. In that study, 3 subjects carried GyrA G93C mutation combined with ParC mutation and failed treatment.[34s] There have been few reports of the GyrA G93C mutation associated with treatment failure; our report adds another example of the strong association of this mutation with high MICs.[39s]

Sitafloxacin combined with doxycycline has been used to treat *Mg* infections that have failed sequential doxycycline-moxifloxacin therapy.[27] In a retrospective review of patients treated with sitafloxacin or moxifloxacin, sitafloxacin cleared 94.2% (95% CI, 87.9%–97.9%) of *Mg* infections that had not previously failed moxifloxacin; this was reduced to 69.5% (95% CI, 60.8%–77.4%) clearance when moxifloxacin had failed previously. Prior failure of moxifloxacin was associated with an 8-fold increased odds of sitafloxacin failure.[35s] There was no difference in *Mg* clearance between sequential monotherapy and combination therapy when patients were stratified by past failure of moxifloxacin.[35s] Another clinical study showed that sitafloxacin-based sequential therapy for infections with *Mg* that possessed the ParC S83I mutation achieved a higher clearance rate than moxifloxacin-based sequential therapy (76% [19 of 25] vs. 47% [14 of 30], $P = 0.03$).[20] Although the clearance of *Mg* in that study, compared with our earlier study,[24] was higher (92% [252 of 274] vs. 67% [12 of 18]), the difference was not significant. One patient who possessed a ParC S83I mutation had failed prior sequential doxycycline-moxifloxacin therapy but cleared *Mg* after sequential doxycycline-sitafloxacin therapy. This may indicate that the site of mutation in the *parC* gene alone cannot accurately predict resistance to sitafloxacin, and that other QRAMs such as *gyrA*

mutations should be considered. A randomized clinical trial comparing sitafloxacin- and moxifloxacin-based sequential treatment for *Mg* infections is currently underway in Japan.[40s] In an area with increasing fluoroquinolone resistance of *Mg*, like China, sitafloxacin may become a preferred option over moxifloxacin when available. However, sitafloxacin has not been used widely in the clinical treatment of *Mg* infection in China.

Concurrent GyrA and ParC S83I mutations increase the risk of sitafloxacin treatment failure[2033s,34s]; clearance of *Mg* that possessed both GyrA and ParC S83I mutations was only 41.7% (10 of 24; 95% CI, 22.1%–63.4%) in Japan.[34s] In that study, combined G93C mutation in GyrA with ParC S83I was present in one subject who failed sequential therapy with doxycycline-sitafloxacin; The G93C mutation in GyrA has been found in other *Mg*-infected persons who failed sitafloxacin treatment,[34s] as did the presence of M95I and D99N mutations in GyrA.[20,33s]

Doxycycline-sitafloxacin sequential therapy was well tolerated. Adverse events associated with sitafloxacin included, in one subject, bilateral Achilles tendon pain, an adverse event reported previously,[21] and in a second subject, muscle weakness that cleared within a week following cessation of sitafloxacin.

Our study has limitations: there were only 22 of 63 *Mg*-infected subjects (35%) available for analysis of primary outcomes; this high rate of loss to follow-up may have implications in determining accurate outcomes. The small number and percentage of potentially eligible subjects enrolled resulted from the rigorous screening process that eliminated subjects with coinfections that required alternative first-line therapies, a common occurrence with *Mg* infections.[14,41s] Furthermore, 2-step sequential therapy may have discouraged the return of certain patients for a third visit: as an example, in the case of 12 subjects who failed to return for TOC because their clinical symptoms had resolved, confirmed remotely. Some patients, who declined enrollment initially, chose other treatments because sitafloxacin has not been widely used for the treatment of *Mg* infections in China. The use of doxycycline sitafloxacin combined therapy has shown no difference in *Mg* clearance between sequential monotherapy and combined therapy of the 2 agents when patients were stratified by past failure of moxifloxacin,[35s] and suggests an approach that may enhance patient compliance.

## CONCLUSION

Our results add early data on the efficacy and tolerability of sequential therapy of doxycycline-sitafloxacin for urogenital *Mg* infections and may provide an option for treatment of *Mg* in China. Monitoring of quinolone resistance–associated mutations in *parC* and *gyrA* genes, in particular, may predict treatment outcomes with sequential therapy that includes sitafloxacin.

## ORCID ID

Xiaohong Su https://orcid.org/0000-0002-2053-7070

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For further references, please see "Supplemental References," http://links.lww.com/OLQ/B157.