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Original Article

Macrolide and fluoroquinolone resistance is uncommon in clinical strains of *Chlamydia trachomatis*☆

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ABSTRACT

We analyzed the 23S rRNA, *gyrA* and *parC* genes of *Chlamydia trachomatis* DNAs from men with urethritis and determined microbiological outcomes of an extended-release azithromycin (azithromycin-SR) regimen (2 g once daily for 1 day) and a sitafloxacin regimen (100 mg twice daily for 7 days) for chlamydial urethritis to clarify the macrolide and fluoroquinolone resistance status of clinical strains of *C. trachomatis*. We amplified the portions of 2 alleles of the 23S rRNA gene and the *gyrA* and *parC* genes from *C. trachomatis* DNAs in 284 first-voided urine specimens from men with chlamydial urethritis by PCR and sequenced their PCR products. We enrolled 369 men with chlamydial urethritis, comprising 314 and 55 treated with the azithromycin-SR regimen and the sitafloxacin regimen, respectively. Alleles 1 and/or 2 of the 23S rRNA gene were analyzed in 162 specimens. No mutations were found in the sequenced regions, including the central portion of domain V. The *gyrA* and *parC* genes were analyzed in 118 and 113 specimens, respectively. No amino acid changes were found within the quinolone resistance-determining region of the *gyrA* gene and in the sequenced region of the *parC* gene. The microbiological outcomes of the azithromycin-SR and sitafloxacin regimens were assessed in 176 and 30 men, respectively. The eradication rates were 96.0% (95% CI 93.1%–98.9%) for the azithromycin-SR regimen and 100% for the sitafloxacin regimen. Clinical strains of *C. trachomatis* with macrolide and/or fluoroquinolone resistance would be uncommon, and azithromycin or fluoroquinolone regimens could be recommended as treatments for chlamydial infections.

1. Introduction

Male non-gonococcal urethritis (NGU) is caused by a variety of microorganisms [1]. *Chlamydia trachomatis* is a predominant pathogen of NGU, and *Mycoplasma genitalium* is another important pathogen [2]. Tetracyclines, macrolides, or fluoroquinolones, which are highly active against *C. trachomatis*, are commonly prescribed to treat patients with NGU. These antimicrobial agents are expected to be effective on *M. genitalium* infections. Recently, however, treatment failures with macrolide or fluoroquinolone regimens have been reported in *M. genitalium* infections [3,4]. In addition, an increase in the prevalence of *M. genitalium* harbouring macrolide and/or fluoroquinolone resistance-associated mutations has been observed [5]. *C. trachomatis* is also exposed to these agents. By contrast, however, drug-resistant *C. trachomatis* has rarely been reported, although a small number of clinical strains of *C. trachomatis* with resistance to tetracyclines, macrolides and/or fluoroquinolones have been isolated [6–10]. In clinical settings, nucleic acid amplification tests are commonly used to detect *C. trachomatis*, but culture is not routinely carried out to isolate *C. trachomatis* from clinical specimens. It would be difficult, therefore, to survey the antimicrobial susceptibility of clinical isolates of *C. trachomatis* periodically, although several surveillances, including a small number of clinical isolates of *C. trachomatis*, have been conducted [10–15]. Owing to the lack of periodic antimicrobial

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susceptibility surveillance of clinical isolates of *C. trachomatis*, the emergence and spread of drug-resistant *C. trachomatis* might have been overlooked. To clarify the status of drug resistance in clinical strains of *C. trachomatis*, we examined *C. trachomatis* DNAs from first-void urine specimens of men with acute urethritis for the presence of macrolide resistance-associated mutations in the 23S rRNA gene and fluoroquinolone resistance-associated mutations in the *gyrA* and *parC* genes [16] and determined the microbiological outcomes in men with acute *C. trachomatis*-positive urethritis treated with a sitafloxacin or extended-release azithromycin (azithromycin-SR) regimen in this study.

2. Materials and methods

2.1. Ethics

This retrospective study was approved by the Institutional Review Board of the Graduate School of Medicine, Gifu University, Gifu, Japan. However, the consent of the subjects was not obtained when this study was conducted because they could not be traced. Therefore, we sufficiently anonymized their information.

2.2. DNA specimens

We enrolled 284 DNA specimens positive for *C. trachomatis* in this study. *C. trachomatis* was detected in first-void urine specimens of men with acute urethritis who visited a urologic clinic (iClinic) in Sendai, Japan, between April 2013 and December 2016 by APTIMA Combo2 (Hologic, Inc., Bedford, MA, USA). The DNA specimens were purified from portions of each first-void urine specimen by QIASymphony DSP Virus/Pathogen Kit (QIAGEN N. V., Hilden, Germany) according to the manufacturer's instruction, and were stored at −70 °C.

2.3. Analyses of the 23S rRNA genes

Portions of alleles 1 and 2 of the 23S rRNA gene, including the central portion of domain V, were amplified from the DNAs in the first-void urine specimens by PCR as previously reported [17]. The PCR products were then sequenced.

2.4. Analyses of the gyrA and parC genes

The portions of the *gyrA* and *parC* genes, including the quinolone resistance-determining region (QRDR) and the analogous region, respectively, were amplified from the DNAs in the first-void urine specimens by PCR with respective primer sets of Ct-*gyrA*-F (5′AAGGACTCTCGTTAGAGTCTC3′) and Ct-*gyrA*-R (5′CGATRCCTGA GGAGCCATTAC3′) for the *gyrA* gene and Ct-*parC*-F (5′GATACAGA AACCCTSGACACAC3′) and Ct-*parC*-R (5′CATGGAAGGTCATGAGAT CCG3′) for the *parC* gene. The PCR products were then sequenced.

2.5. Microbiological outcomes in men with acute C. trachomatis-positive urethritis treated with the azithromycin or sitafloxacin regimen

We retrospectively retrieved the clinical data of men complaining of acute urethritis symptoms who visited the iClinic between January 2013 and June 2017. We enrolled 369 men whose first-void urine specimens were positive for *C. trachomatis* by APTIMA Combo2 at their first visits. Of these men, 314 were treated by an azithromycin-SR regimen (2 g once daily for 1 day), and 55 were treated by a sitafloxacin regimen (100 mg twice daily for 7 days). Microbiological outcomes of the antimicrobial chemotherapies were assessed based on the results of post-treatment test of APTIMA Combo2 for *C. trachomatis* 21–60 days after the initiation of treatment.

2.6. Analyses of ribosomal proteins in C. trachomatis from men with azithromycin-SR treatment failures

The portions of the genes encoding L4 and L22 ribosomal proteins, including the regions corresponding to Q62-G66 in L4 and R88-A93 in L22 of *Escherichia coli*, which were closest to the macrolide binding site, were amplified from the DNAs in the first-void urine specimens by PCR as previously reported [17]. The PCR products were then sequenced.

3. Results

3.1. Mutations in the 23S rRNA gene

Allele 1 and/or 2 of the 23S rRNA gene was amplified by PCR, and their PCR products were sequenced in 162 of 284 DNA specimens (Fig. 1). No mutations were found in the sequenced regions of allele 1 and allele 2, including A2058, A2059 and T2611 (according to *E. coli* numbering) (Table 1).

3.2. Amino acid changes in GyrA and ParC

The QRDR of the *gyrA* gene and the analogous region of the *parC* gene were analyzed in the 162 DNA specimens, from which at least one allele of the 23S rRNA gene could be sequenced (Fig. 1). The *gyrA* and *parC* genes were analyzed in 118 and 113 specimens, respectively. Amino acid changes of V61A and H129Q were found in the sequenced region of the *gyrA* gene in 44 DNA specimens but were not localized within the QRDR of the *gyrA* gene (Table 1). No amino acid changes were found in the sequenced region of the *parC* gene.

3.3. Microbiological outcomes of antimicrobial chemotherapies

We enrolled 314 men treated with the azithromycin-SR regimen and 55 men treated with the sitafloxacin regimen in this study. Microbiological outcomes of the azithromycin-SR and sitafloxacin regimens were ultimately assessed in 176 (56.1%) and 30 (54.5%) men, respectively (Fig. 2). The eradication rates of *C. trachomatis* were 96.0% (95% CI 93.1%–98.9%) for the azithromycin-SR regimen and 100% for the sitafloxacin regimen.

3.4. Ribosomal proteins in C. trachomatis from men with azithromycin-SR treatment failures

Of the 7 men in whom *C. trachomatis* persisted after treatment with the azithromycin-SR regimen, the pre-treatment DNA specimens of 5 men were stored (Table 2). These 5 specimens were examined for mutations of the 23S rRNA gene and for the ribosomal proteins of L4 and L22. In 3 and 5 specimens, the genes of L4 and L22 were respectively amplified by PCR and sequenced. No amino acid changes in L4 were found in the 3 specimens, whereas G52S, R65C, and V77A in L22 were found in 3 of the 5 specimens.

4. Discussion

We found no mutations in the 23S rRNA gene of *C. trachomatis* in the DNA specimens derived from *C. trachomatis*-positive first-void urine specimens of the men with acute urethritis. However, one study in Russia reported that 4 strains of *C. trachomatis*, for which MICs of erythromycin, azithromycin and josamycin were >5.12 μg/mL, were isolated from 3 patients with chronic salpingitis,

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