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Original Article

Antimicrobial susceptibilities of *Chlamydia trachomatis* isolated from the urethra and pharynx of Japanese males

Seiji Kai [a, b, e], Koichiro Wada [a, e, *], Takuya Sadahira [a, e], Motoo Araki [a, e], Ayano Ishii [a, e], Toyohiko Watanabe [a, e], Koichi Monden [c, e], Satoshi Uno [d, e], Tohru Araki [c, e], Yasutomo Nasu [a, e]

[a] Department of Urology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama, 700-8558, Japan
[b] Department of Urology, Hiroshima City Hiroshima Citizens Hospital, 7-33, Moto-machi, Naka-ku, Hiroshima, 730-8518, Japan
[c] Araki Urology Clinic, 390-1 3F, Sasaoki, Kurashiki, 710-0834, Japan
[d] Hirashima Clinic, 1041-4, Higashi-hirashima, Higashi-ku, Okayama, 709-0631, Japan
[e] Okayama Urological Research Group (OURG), 2-5-1, Shikata-cho, Kita-ku, Okayama, 700-8558, Japan

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ABSTRACT

Objectives: Sexually transmitted infections due to *Chlamydia trachomatis* (*C. trachomatis*) are a worldwide public health problem. The aim of this study was to investigate the drug susceptibilities of *C. trachomatis* strains isolated from the urethra and pharynx of Japanese males.
Methods: Urethral and pharyngeal swabs were collected between 2013 and 2014 from Japanese males with urethritis. Using a McCoy cell line, 18 chlamydial strains were isolated from urethra in 18 patients and 7 from the pharynx in 7 of the 18 patients. The minimum inhibitory concentrations (MICs) of levofloxacin (LVFX) and azithromycin (AZM) were measured using the standard method of the Japanese Society of Chemotherapy.
Results: The MICs of LVFX and AZM against urethral chlamydial strains were 0.125–0.5 μg/mL and 0.125–0.25 μg/mL, respectively. In pharyngeal strains, the MICs of LVFX and AZM were 0.125–0.25 μg/mL and 0.125–0.25 μg/mL, respectively. In 7 patients with chlamydial strains isolated from both the urethra and pharynx, the MICs of LVFX between these strains were identical in 3 of 6 patients (no growth was observed for one pharyngeal strain), while the MICs of AZM between these strains were identical in all 6 patients (not performed for one patient).
Conclusions: Our data suggest that *C. trachomatis* strains isolated from the urethra and pharynx of Japanese males are susceptible to LVFX and AZM. Although measuring the MICs of chlamydial strains is labor intensive, it is a significant surveillance tool for treating chlamydial infections and preventing the spread of STIs.

1. Introduction

The spread of sexually transmitted infections, particularly male urethritis and uterine cervicitis due to *Chlamydia trachomatis* (*C. trachomatis*), is a major worldwide health concern [1–3]. In Japanese guidelines published between 2014 and 2016, macrolides, tetracyclines and fluoroquinolones are the recommended treatment for genitourinary tract infections due to *C. trachomatis* [4,5]. However, male urethritis and cervicitis are also transmitted from the pharynx during oral sex [6,7]. Several studies have reported that pharyngeal chlamydial infection is refractory to some antimicrobial regimens [8–11]. Since one of the reasons might be a low penetration rate of antimicrobial agents into the pharynx [11], there is the possibility that the lower drug susceptibility of *C. trachomatis* cannot be overcome. However, while drug-resistant strains of *C. trachomatis* have been observed in other countries [12–15], there have also been reports of high drug susceptibilities

* Corresponding author. 2-5-1, Shikata-cho, Kita-ku, Okayama, 700-8558, Japan. Fax: +81 86 231 3986.
E-mail address: gmd17055@s.okadai.jp (K. Wada).

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in strains isolated from the urethras of Japanese males [16]. The aim of this study is to investigate the drug susceptibilities of *C. trachomatis* strains isolated from both the urethra and the pharynx of Japanese males. The target drugs, levofloxacin and azithromycin, are recommended in the treatment guidelines and are the most frequently administered antimicrobials by clinicians for *C. trachomatis* infection in Japan.

2. Materials and methods

Specimens were obtained from male patients diagnosed with urethritis or patients who wanted to check for the presence of urethritis at Okayama University Hospital, Araki Urology Clinic and Hirashima Clinic located in Okayama, Japan between 2013 and 2014.

2.1. Patient characteristics

Patient characteristics were ascertained from the medical records of patients for whom *C. trachomatis* was isolated from the urethra or pharynx. The following characteristics were noted: age, presence of symptoms caused by urethritis, and results of nucleic acid amplification testing of urine samples. Antimicrobial administration and treatment outcome were not included because the objective of this study was to survey for the presence of drug-resistant *C. trachomatis* strains.

2.2. Clinical specimens and chlamydial cultures for isolation of C. trachomatis

A cotton swab (Copan Diagnostics Inc., Italy) was inserted into throat and swab the pharynx. For urethra, a cotton swab was gently inserted about 3 cm into the urethra and gently rotated. Each swab was placed in a tube containing 0.5 mL of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) • NaOH buffer containing sucrose (0.075 g/ml) and L-glutamic acid (0.72 mg/ml) buffer with micro beads followed by preservation at −70 °C until cultured for *Chlamydia* spp.

We performed chlamydial culture of clinical swab specimens using a previously described method [17]. Frozen tubes containing swabs were quickly thawed in a water tank set at 37 °C, after which the tubes were stirred using a vortex mixer to release the epithelial cells and chlamydial organisms from the cotton swab. The epithelial cells were sonicated using the Bioruptor® UCD-200T Ultrasonic Wave Disruption System (Cosmo Bio Co. Ltd., Tokyo, Japan). Following centrifugation at 300 ×*g* for 3 min at room temperature, 0.25 mL of supernatant was placed on McCoy cells that had been cultured as confluent monolayers in a 24-well cell culture plate (Corning Costar Corp., Corning, NY, USA). The plate was centrifuged (860 ×*g*, 25 °C, 60 min) using a Hitachi himac CR21E centrifuge (Hitachi Koki Co. Ltd., Tokyo, Japan) to adhere chlamydial organisms to epithelial cells. One mL of Dulbecco's modified Eagle medium (DMEM; Nissui, Tokyo, Japan) including 1 μg/mL of cycloheximide, 10 μg/mL of kanamycin, 10 μg/mL of vancomycin, 10 μg/mL of amphotericin B and supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL, Life Technologies Inc., Grand Island, NY, USA) was added to each well, and the inoculated cells were incubated at 37 °C in 5% CO_2. Cell conditions were monitored, at appropriate intervals using a phase-contrast microscope, for evidence of the cytopathic effect. Immediately following observation of cell bursts, the cells were removed from the plates with sterile rubber fragments, suspended in 1 mL/well of sucrose-phosphate-glutamate (SPG) buffer and preserved at −70 °C.

2.3. Fluorescent staining

Fluorescent staining to observe chlamydial inclusion was performed as previously described [17]. McCoy cell monolayers prepared on a cover glass (14 mm in diameter) were stained 48–50 h post-inoculation, during the preservation on incubated McCoy cells detailed in the previous section. The cells were fixed with 99.5% ethanol and stained with fluorescein-conjugated monoclonal antibody directed against a genus-specific antigen (*Chlamydia* FA Seiken [DFA stain]; Denka Seiken, Tokyo, Japan) Matsumoto et al. [18].

2.4. Drug susceptibility testing

Drug susceptibility testing was performed according to the standard method of the Japanese Society of Chemotherapy [19]. Two antimicrobial agents, levofloxacin (LVFX; Sigma–Aldrich Japan Co. LLC, Tokyo, Japan) and azithromycin (AZM; LKT Laboratories, Inc., USA), which are recommended in treatment guidelines [4,5] were chosen for susceptibility testing.

2.5. Preliminary experiments

Preliminary experiments using standard strains of *C. trachomatis*, including a reference strain, were performed to evaluate the quality of the HeLa229 cell line and the drug-susceptibility measuring system [19]. HeLa229 cells purchased from the National Institute of Infectious Diseases were cultured as described above in DMEM containing 10% heat-inactivated FBS. Cell conditions were monitored at appropriate intervals using a phase-contrast microscope. Chlamydial strains used in these preliminary experiments included serovar A, C, D/UW-3/Cx (reference strain), F, G and H. The MICs of LVFX and AZM against these strains were measured before testing of the clinical isolates.

2.6. Ethics

This clinical study was approved by the Okayama University Institutional Review Board prior to study initiation (Registration no.; 1519). The study was registered with the University Hospital Medical Information Network (UMIN), Japan (Registration no.; R000027274). Participants reviewed the informed consent document and received individual counseling with a thorough discussion as to alternative treatment, including nonparticipation.

3. Results

3.1. Patient characteristics

A total of 18 patients diagnosed as urethritis due to *C. trachomatis* were picked up from our database and enrolled in this study. The mean age of the 18 patients was 25.7 ± 7.8 years. Symptoms included micturition pain in 8 patients, pus discharge in 4 patients, both in 4 patients and none in 1 patient. Pharyngitis symptomology was not observed. All patients were diagnosed with chlamydial urethritis by polymerase chain reaction (PCR) or standard displacement amplification (SDA). During the 18 patients, single-dose AZM 2000 mg were administered for 10 patients, single-dose AZM 1000 mg for 6 patients and once-daily 500 mg LVFX for 7 or 14 days for 2 patients. Cure in 14 patients out of 18 have been confirmed using their urine samples, however, examination using urine samples after antimicrobial administration were not performed in 4 patients. Because of residual symptoms, once-daily 500 mg LVFX for 7 days were additionally administered for 2 patients; 1 with single-dose AZM 2000 mg (Patient No. 10), 1 with