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In Vitro Activities of Several Antimicrobial Agents against Recently Isolated and Genotyped *Chlamydia trachomatis* Urogenital Serovars D through K[▽]

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A systematic evaluation of the susceptibility of all *Chlamydia trachomatis* urogenital serovars (D through K) to levofloxacin, erythromycin, doxycycline, clarithromycin, and azithromycin was performed. All *C. trachomatis* serovars had comparable susceptibilities with respect to the various antimicrobials tested, thus confirming the homogeneous data so far obtained regarding the susceptibility of *C. trachomatis* to antimicrobial agents.

Infections caused by *Chlamydia trachomatis* serovars D through K are among the most prevalent causes of urogenital diseases worldwide (4). Although chlamydial infections are often asymptomatic, they can cause long-term sequelae, including pelvic inflammatory disease, chronic pelvic pain, and tubal factor infertility (5).

In the past, doxycycline has been regarded as the first-choice antibiotic therapy for *C. trachomatis* genital infections (10) but is subject to problems of compliance (1). Subsequently, a variety of broad-spectrum antibiotics, including fluoroquinolones and macrolides, have been recommended for the treatment of pelvic inflammatory disease associated with *C. trachomatis* (3, 7).

The 2006 Centers for Disease Control and Prevention (CDC) sexually transmitted disease (STD) treatment guidelines (2) indicated doxycycline and azithromycin as recommended agents against chlamydial urogenital infection and erythromycin, levofloxacin, and ofloxacin as alternative drugs.

So far, few studies have evaluated the susceptibilities of the various *C. trachomatis* serovars to antimicrobial agents (8). This study was undertaken to evaluate the *in vitro* activity of several antimicrobial agents known to be active against *C. trachomatis* in order to test the sensitivity of all *C. trachomatis* serovars (D through K) associated with nondisseminating sexually transmitted disease. The antimicrobial drugs used were levofloxacin, erythromycin, doxycycline, clarithromycin, and azithromycin.

A total of 50 *C. trachomatis* strains were tested. Of these, 5 were reference strains (Ic Cal-8 serovar D, Bour serovar E, 392-F serovar G, 580 serovar H, and UW-36 serovar G) and 45 were strains isolated from urethral swabs obtained from male patients with nongonococcal urethritis or from women with cervicitis, isolated in our laboratory at S. Orsola University Hospital, Bologna, Italy, over the years 2005 and 2006. About 65% of the patients were from Italy, 24% from East Europe, 4% from Africa, 3% from America, 2% from West Europe, and 2% from Asia.

C. trachomatis serovars were identified by using amplification and sequencing of the major outer membrane protein gene (*ompA*). DNA of all 45 *C. trachomatis* isolates and 5 reference strains, grown in LLC-MK2 cells, was extracted for molecular analysis employing a commercially available kit (tissue kit; Qiagen, Düsseldorf, Germany) and used as a template for amplification of a 1,050-bp chlamydial *ompA* gene fragment. The reaction was performed according to the method of Lan et al. (6). The amplicons were purified using a Wizard purification kit (Promega, WI), and each PCR product was sequenced twice in each direction (Bio-Fab Research, Rome, Italy). The nucleotide sequences were compared with the same regions of the *C. trachomatis* reference serovars in the GenBank database using the BLAST at the National Center for the Biotechnology Information (www.ncbi.nlm.nih.gov/).

From the analyses of the sequencing data, 8 *C. trachomatis* serovars, deduced from genotypes, were found in the following order of prevalence among the 45 *C. trachomatis* strains isolated: 9 serovar D, 8 serovar G, 7 serovar E, 7 serovar F, 4 serovar I, 4 serovar J, 4 serovar K, and 2 serovar H strains. The molecular typing of the five reference strains (two *C. trachomatis* type D, one type E, one type J, and one type H) confirmed the previous serotyping.

The antimicrobial drugs levofloxacin (GlaxoSmithKline, Verona, Italy), doxycycline, erythromycin, azithromycin (Sigma, Milan, Italy), and clarithromycin (Abbott, Latina, Italy) were provided as powders and solubilized according to the instructions of manufacturers. Antimicrobial susceptibility testing was performed with LLC-MK2 cells grown in 24-well plates with Eagle's minimum essential medium. Each of the 24-well plates was inoculated with 5×10^3 inclusion-forming units (IFU) per milliliter. After centrifugation at $1,700 \times g$ for 1 h, the medium was removed and replaced with medium containing different concentrations of antimicrobial drugs (3). After incubation at 35°C for 48 h, infected monolayers were washed with phosphate-buffered saline (PBS), fixed with methanol, and stained for inclusion with a fluorescein-conjugated monoclonal anti-

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TABLE 1. *In vitro* MICs and MBCs of five antimicrobial drugs against 50 *C. trachomatis* strains

Serovar (no. of isolates)	Concn (μg/ml)									
	Levofloxacin		Erythromycin		Doxycycline		Clarithromycin		Azithromycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
D (9)	0.5	0.5	0.5–1.0	1.0–2.0	0.03–0.06	0.06–0.125	0.03	0.03–0.06	0.25	0.5–1.0
D (1)[a]	0.5	0.5	0.5	1.0	0.03	0.06	0.03	0.06	0.25	1.0
D (1)[a]	0.5	0.5	1.0	2.0	0.06	0.06	0.06	0.125	0.25	1.0
E (7)	0.5	0.5	0.5–1.0	1.0–2.0	0.03–0.06	0.06–0.125	0.015–0.06	0.03–0.125	0.25–0.5	1.0
E (1)[a]	0.5	0.5	0.5	2.0	0.03	0.06	0.03	0.06	0.25	1.0
F (7)	0.5	0.5	0.5–1.0	1.0–2.0	0.03–0.06	0.06–0.125	0.015–0.06	0.03–0.125	0.5	0.5–1.0
G (8)	0.25–0.5	0.25–1.0	0.5–1.0	1.0–2.0	0.03–0.06	0.06–0125	0.03–0.06	0.06–0.125	0.5	1.0
H (2)	0.5	0.5	0.5–1.0	1.0–2.0	0.03–0.06	0.06–0.125	0.03	0.06	0.25	1.0
H (1)[a]	0.5	0.5	1	2.0	0.06	0.125	0.06	0.125	0.25	1.0
I (4)	0.5	0.5	0.5–1.0	1.0–2.0	0.06	0.06–0.125	0.015–0.06	0.03–0.125	0.5	1.0
J (4)	0.5	0.5–1.0	1.0	2.0	0.06	0.06–0.125	0.03	0.06–0.125	0.25	0.5
J (1)[a]	0.5	0.5	0.5	1.0	0.06	0.125	0.06	0.125	0.25	0.5
K (4)	0.5	0.5	0.5	1.0	0.03–0.06	0.06–0.125	0.015–0.06	0.03–0.125	0.5	0.5–1.0

[a] Reference strains.

body specific for the chlamydial lipopolysaccharide genus-specific antigen (Meridian Diagnostics, Inc., Cincinnati, OH). The MIC was defined as the lowest concentration that reduced the number of inclusions more than 90%, compared with the level for drug-free controls. The minimal bactericidal concentration (MBC) was measured by aspirating the antibiotic-containing medium, washing the monolayer twice with PBS, and incubating it in growth antibiotic-free medium for 48 h at 35°C (3). Cells were fixed and chlamydial inclusions were stained as described above (3). The MBC was the lowest concentration of the drug reducing more than 90% demonstrable inclusions after monolayers were reincubated in antimicrobial-free medium. All tests were run in triplicate.

The MICs and MBCs of the antimicrobial agents are reported in Table 1. The MICs of levofloxacin against *C. trachomatis* serovars ranged between 0.25 and 0.5 μg/ml, whereas the MBCs ranged between 0.25 μg/ml and 1.0 μg/ml. The MICs of erythromycin ranged between 0.5 μg/ml and 1.0 μg/ml, whereas the MBCs ranged between 1.0 μg/ml and 2.0 μg/ml. Doxycycline showed MIC values ranging between 0.03 μg/ml and 0.06 μg/ml, whereas the MBCs ranged between 0.06 μg/ml and 0.125 μg/ml. The MICs of clarithromycin ranged between 0.015 μg/ml and 0.06 μg/ml, whereas the MBCs ranged between 0.03 μg/ml and 0.125 μg/ml. Finally, azithromycin showed MIC values ranging between 0.25 μg/ml and 0.5 μg/ml, whereas the MBCs ranged between 0.5 μg/ml and 1.0 μg/ml. All agents except levofloxacin were bactericidal by MBC at 1 to 2 times the MIC. No differences have been detected in tests run in triplicate.

The results of this *in vitro* study confirm the data obtained by Welsh et al. (11) related to the activity of tetracycline, azithromycin, and erythromycin against only 5 urogenital serovars of *C. trachomatis*, showing MBCs higher than the MIC values in most cases. In addition, we reported data similar to those obtained by Roblin et al. (9) in relation to the higher *in vitro* activity of clarithromycin than of erythromycin.

So far, the studies of the susceptibility of *C. trachomatis* to antimicrobial agents usually tested untyped strains. The present study is, to our knowledge, the first one where numerous and recently isolated strains from all eight urogenital serovars were typed and tested. The results demonstrate that all *C. trachomatis* serovars (D through K) had comparable susceptibilities with respect to the various classes of antimicrobials tested, thus confirming the homogeneous data so far obtained regarding the susceptibility of *C. trachomatis* to antimicrobial agents.

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