

## Susceptibilities of *Chlamydia trachomatis* Isolates Causing Uncomplicated Female Genital Tract Infections and Pelvic Inflammatory Disease

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**The in vitro susceptibilities of 45 recent clinical isolates of *Chlamydia trachomatis* obtained from women with asymptomatic genital tract infection, mucopurulent cervicitis, or pelvic inflammatory disease to doxycycline, azithromycin, ofloxacin, and clindamycin were determined. In addition, susceptibilities of 12 isolates to amoxicillin and trimethoprim-sulfamethoxazole were also determined. Isolates also were serotyped with a panel of monoclonal antibodies specific for chlamydial major outer membrane protein; 24 of 45 (53%) belonged to serovars Ia and E. For all isolates, the MIC range of doxycycline was 0.008 to 0.06 µg/ml, for trimethoprim-sulfamethoxazole it was 0.03 to 0.25 µg/ml, for azithromycin it was 0.125 to 2.0 µg/ml, for ofloxacin it was 0.5 to 1.0 µg/ml, for clindamycin it was 0.25 to 2.0 µg/ml, and for amoxicillin it was 0.25 to 4.0 µg/ml. The ranges of minimum chlamydiae concentrations were generally 1 to 4 dilutions above the MICs of most agents, with a rank order similar to those of the MICs. Comparing the minimum chlamydiae concentrations for 90% of isolates tested, isolates causing asymptomatic infection belonged to a greater variety of serovars and were relatively more susceptible to doxycycline and azithromycin than isolates causing mucopurulent cervicitis or pelvic inflammatory disease; these differences in susceptibility were not detected among the other study agents. These data indicate that additional studies are needed to better define the apparent association of certain chlamydial serovars with the clinical severity of disease and the in vitro susceptibilities to certain antimicrobial agents.**

*Chlamydia trachomatis* is a highly prevalent and important cause of sexually transmitted disease (STD) among women of childbearing age, accounting for an estimated 4 million infections in the United States each year (3). The clinical spectrum of female genital tract infections caused by this pathogen ranges from asymptomatic infection to mucopurulent cervicitis (MPC), pelvic inflammatory disease (PID), and perihepatitis (3). The long-term sequelae and costly complications of chlamydial infections of the female genital tract include tubal scarring, infertility, ectopic pregnancy, and chronic pelvic pain (3). Therefore, early detection and treatment of genital tract infections in women are extremely important components of current chlamydia prevention and control program efforts (3, 4).

The Centers for Disease Control and Prevention (CDC) provides recommendations for the prevention, diagnosis, and treatment of female genital tract infections caused by *C. trachomatis* (3, 4). Tetracycline and its derivatives, e.g., doxycycline, have been the mainstay of antichlamydial therapy in adults for many years and continue to be recommended by the CDC as a primary therapy for infections in adults (4). Erythromycin and, more recently, azithromycin have been recommended as alternatives for the therapy of adults allergic to or unable to tolerate tetracyclines (4). Studies have shown that in vitro azithromycin is at least as active if not slightly more active than erythromycin against *C. trachomatis* (12). However, azithromycin is not recommended for the treatment of chlamydial infections in pregnant or breastfeeding women (4).

Newer fluoroquinolones such as ofloxacin and older agents such as clindamycin, amoxicillin, and certain sulfonamides have also been included in CDC STD treatment guidelines (4). These alternative regimens have previously been shown to differ in their antichlamydial in vitro inhibitory activities as well as in their side effects and toxicities (1, 4-6, 8, 9). Thus, the goal of the study described here was to assess the in vitro inhibitory and cidal activities of agents currently recommended or frequently prescribed for the treatment of chlamydial genital tract infections, including PID, in women and to compare their activities in relation to the severity of clinical disease.

A total of 45 clinical isolates were obtained from women with diagnoses of chlamydial infection, ranging from asymptomatic cervical infection ( $n = 20$ ) to MPC ( $n = 10$ ) and PID ( $n = 15$ ). Chlamydial isolates from patients with all categories of infection were geographically and temporally related. Isolates were serotyped by using a panel of monoclonal antibodies (MAbs) directed against chlamydial major outer membrane protein epitopes; MAbs were obtained as a Micro-IF kit from the Washington Research Foundation, Seattle, Wash., and chlamydial serotyping was performed as described previously (11).

The following antimicrobial agents were obtained as standard powders for in vitro susceptibility testing from the indicated sources and were reconstituted according to the manufacturers' instructions: amoxicillin (Beecham Laboratories, West Point, Pa.), azithromycin and doxycycline (Pfizer Laboratories, Groton, Conn.), clindamycin (The Upjohn Company, Kalamazoo, Mich.), ofloxacin (Ortho Pharmaceuticals, Raritan, N.J.), and trimethoprim-sulfamethoxazole (TMP-SMX; Hoffmann-La Roche Inc., Nutley, N.J.).

Chlamydial cells were propagated in McCoy cell culture by

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TABLE 1. In vitro susceptibilities to selected antimicrobial agents, of *C. trachomatis* isolates obtained from women with genital tract infections and PID

Agent	Concn ( $\mu\text{g/ml}$ ) for isolates from women with clinical diagnoses of <sup>a</sup> :					
	Asymptomatic cervical infection ( $n = 20$ )		MPC ( $n = 10$ )		PID ( $n = 15$ )	
	Range	90% Inhibition <sup>b</sup>	Range	90% Inhibition	Range	90% Inhibition
Doxycycline						
MIC	0.008–0.03	0.03	0.03	0.03	0.015–0.06	0.06
MCC	0.015–0.25	0.03	0.03–4.0	0.25	0.015–4.0	0.25
Azithromycin						
MIC	0.125–1.0	0.5	0.25–1.0	1.0	0.25–2.0	0.5
MCC	0.5–1.0	1.0	0.5–4.0	4.0	0.5–>4.0	4.0
Ofloxacin						
MIC	0.5–1.0	0.5	0.5–1.0	0.5	0.5–1.0	0.5
MCC	0.5–2.0	2.0	0.5–4.0	4.0	1.0–>4.0	4.0
Clindamycin						
MIC	0.25–2.0	2.0	0.5–2.0	2.0	0.25–2.0	2.0
MCC	1.0–2.0	2.0	1.0–4.0	2.0	1.0–>4.0	4.0
TMP-SMX						
MIC	0.06–0.25	0.06	0.03–0.125	0.06	0.03–0.125	0.06
MCC	0.06–0.5	0.5	0.125–4.0	1.0	0.25–4.0	1.0
Amoxicillin						
MIC	0.25–1.0	0.5	0.5–4.0	1.0	0.25–2.0	1.0
MCC	0.5–2.0	2.0	1.0–>4.0	>4.0	0.5–2.0	2.0

<sup>a</sup> Twelve isolates were tested.

<sup>b</sup> 90%, drug concentration inhibitory or cidal for 90% of chlamydial isolates tested in each group of clinical diagnostic severity.

previously published procedures (2), and in vitro susceptibility testing was performed as described previously (7, 10), with minor modifications. The MIC of each agent was defined as the concentration of drug at which no typical inclusions were identified on direct fluorescent antibody staining after the first passage in McCoy cell culture. The minimum chlamydiae concentration (MCC) was defined as the lowest concentration of drug that permitted no inclusions to be formed upon second passage in antimicrobial agent-free medium. Briefly, chlamydial isolates were inoculated into 48-well microtiter plates (48-well tissue culture clusters; Costar, Cambridge, Mass.) containing McCoy cells. Microtiter plates were centrifuged at  $1,750 \times g$  for 1 h, and the contents of the wells were subsequently aspirated. Antimicrobial agents were added to each well in twofold dilutions ranging from 0.008 to 4.0  $\mu\text{g/ml}$  in Eagle's minimum essential medium containing 0.003 mM glucose per ml, 1  $\mu\text{g}$  of cycloheximide per ml, 1% L-glutamine (200 nM solution), and 10% fetal calf serum. The plates were then incubated at 37°C in 5% CO<sub>2</sub> for 48 h. After the initial incubation period, the wells were fixed with methanol and were stained with a genus-specific MAb reagent (Pathfinder; Kallestad Diagnostics, Austin, Tex.) for the identification of inclusions to determine the MIC. A subsequent passage was performed in antimicrobial agent-free medium to define the MCC.

The results of in vitro susceptibility testing of all study drugs against representative isolates are summarized in Table 1. Doxycycline was the most active; this was followed by TMP-SMX, azithromycin, ofloxacin, amoxicillin, and clindamycin. MCCs were generally 1 to 4 dilutions higher than MICs of most agents. For some agents such as ofloxacin and TMP-SMX a consistent trend toward greater differences in the MICs for 90% of isolates tested (MIC<sub>90s</sub>) and the MCCs for 90% of

isolates tested (MCC<sub>90s</sub>) were observed among all groups. Comparing MCC<sub>90s</sub>, chlamydial isolates recovered from women with asymptomatic infections were relatively more susceptible in vitro to doxycycline and azithromycin than were isolates from women with MPC and PID (Table 1). The MIC<sub>90</sub> and MCC<sub>90</sub> ratios of these agents for isolates that caused symptomatic infections versus those that caused MPC and PID were also higher. Differences corresponding to the severity of clinical disease were not apparent for the other agents. However, a greater variety of chlamydial serovars was isolated from women with asymptomatic infections than from women with MPC or PID. The overall serovar distribution by category of diseases is given in footnote a of Table 2. The susceptibilities of isolates according to serovar are given in Table 2 by category of infection. In the present study, serovars Ia, E, F, D, and D<sup>-</sup> accounted for 36 of 45 (80%) strains isolated from patients with all types of infection including PID, but serovar Ia predominated among women diagnosed with MPC and PID. Regardless of the clinical severity category of chlamydial infection, the highest MCCs of doxycycline, azithromycin, ofloxacin, and clindamycin were detected among strains belonging to serovar Ia (Table 2). The overall differences in the susceptibilities of chlamydial isolates from women with asymptomatic infection versus the susceptibilities of those from women with PID or MPC were statistically significant or approached significance for amoxicillin, TMP-SMX, and doxycycline. Also, the distribution of serovars by category of disease did not differ significantly. However, the overall number of isolates was small.

Despite major advances in screening and diagnostic technologies and an expanding array of therapeutic agents, *C. trachomatis* continues to be an important cause of genital tract infections in women of childbearing age (3, 7). Tetracyclines,

TABLE 2. Susceptibilities to selected antimicrobial agents of 45 isolates representing chlamydial serovars causing female genital tract infections and PID

Serovar (no. of isolates) <sup>a</sup>	Concn range (µg/ml)							
	Doxycycline		Azithromycin		Ofloxacin		Clindamycin	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
B (2)	0.015	0.015	0.125	0.5	0.25	0.5	0.25–1.0	2.0
D/D <sup>-</sup> (6)	0.015–0.3	0.03	0.125–0.25	0.5	0.5	0.5	0.5–2.0	1.0–2.0
E (9)	0.015–0.03	0.03	0.25–0.5	0.5–1.0	0.25–0.5	0.5–1.0	0.5–1.0	1.0–4.0
F (6)	0.03	0.03–0.06	0.25–0.5	0.5	0.5–1.0	1.0–2.0	0.5–1.0	1.0–2.0
Ia (15)	0.03–0.125	0.03–4.0	0.5–1.0	0.5–>4.0	0.5–1.0	1.0–>4.0	1.0–2.0	1.0–>4.0
K (2)	0.015	0.015	0.125	0.25	0.25	0.5	0.25	1.0
J (5)	0.015–0.03	0.015–4.0	0.5	0.5–1.0	0.5	0.5–4.0	1.0–2.0	1.0–>4.0

<sup>a</sup> The serovar distribution by disease category was as follows: asymptomatic infections, B, D/D<sup>-</sup>, K (2 isolates each), E (4 isolates), F (3 isolates), Ia (6 isolates), and J (1 isolate); PID or MPC, D/D<sup>-</sup>, J (4 isolates each), E (5 isolates), F (3 isolates), and Ia (9 isolates).

specifically doxycycline, remain the most active agents among the available therapeutic regimens for chlamydial infections. CDC has recommended the use of tetracyclines and newer azalide derivatives such as azithromycin in the most recent guidelines for the treatment of chlamydial infections (4). However, the side effect of gastrointestinal intolerance produced by tetracyclines has been a major problem that may discourage patients from taking these medications (4). Azithromycin, a drug which has extended tissue penetration, half-life, and efficacy equivalent to those of tetracyclines when it is given as a single 1-g oral dose, is a promising alternative antichlamydial agent (4, 12). The use of other alternative agents such as ofloxacin has also been recommended in recent CDC STD treatment guidelines, and their use for empiric therapy of genital tract infections and PID is increasing (4). However, in pregnant women, tetracyclines, fluoroquinolones, and the newer azalide derivative azithromycin are contraindicated; thus, erythromycin and amoxicillin remain the principle alternatives for therapy of infections during pregnancy (4). Sulfonamides, such as TMP-SMX and sulfisoxazole, are no longer recommended as primary therapeutic regimens for chlamydial infections of the genital tract but are frequently used to treat nonspecific urethritis and other infections of the female genitourinary tract (7).

The results of the present study suggest that, at least in vitro, the inhibitory activities of the therapeutically relevant antichlamydial agents are not always equivalent to bactericidal activities. The data suggest that, at least in vitro, differences in susceptibilities to a variety of agents correlated with the serovar of the strain; thus, the serovars may serve as a phenotypic marker for susceptibility. In vitro differences in the relative susceptibilities of chlamydial isolates causing asymptomatic infections versus those causing symptomatic infections are interesting but appear to be influenced by the predominance of specific serovars in the populations of strains included in the study. However, these findings represent novel observations

that may be important in the development of future public health recommendations for the prevention and treatment of chlamydial infections of the genital tract in women and especially in those with PID. Additional investigations involving larger patient populations and a greater diversity of clinical isolates are needed.

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