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In Vitro Susceptibilities of *Chlamydia pneumoniae* (*Chlamydia* sp. Strain TWAR)

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The in vitro susceptibilities of two clinical isolates of *Chlamydia pneumoniae* from Brooklyn, N.Y., were determined for tetracycline, erythromycin, sulfamethoxazole, ciprofloxacin, and three new macrolides—azithromycin, clarithromycin, and roxithromycin. Clarithromycin was the most active drug tested, followed by the other macrolides, tetracycline, and ciprofloxacin.

Chlamydia pneumoniae, previously designated *Chlamydia* sp. strain TWAR, has been associated with respiratory disease, particularly pneumonia, in several reports from various geographic areas (2, 4, 6). While *C. pneumoniae* has been estimated to cause 6 to 12% of community-acquired pneumonia in some populations, little information exists regarding response to specific antimicrobial therapy (2).

In the only previous report of in vitro susceptibility of *C. pneumoniae*, all respiratory isolates were from a single geographic area (Seattle, Wash.), with an additional ocular isolate from Taiwan (TW-183) (3). We recently isolated *C. pneumoniae* from two patients hospitalized with pneumonia in Brooklyn, N.Y. The in vitro antimicrobial susceptibilities of these two isolates (designated 2023 and 2043) were assessed in parallel with TW-183 (obtained from the Washington Research Foundation, Seattle). Both clinical isolates were obtained from nasopharyngeal swab specimens and grown up in HeLa 229 cells. Each isolate had been passed eight times before susceptibility testing. Species identification was confirmed by positive immunofluorescent staining with *C. pneumoniae*-specific monoclonal antibody (obtained from the Washington Research Foundation).

In the previous report the antimicrobial agents most active against *C. pneumoniae* were erythromycin and tetracycline (3). Several quinolones and newer macrolides have good activity against *C. trachomatis* (1, 5), and we investigated some of these agents for their activity against *C. pneumoniae*.

The antimicrobial agents tested were tetracycline (Lederle Laboratories, Pearl River, N.Y.), erythromycin gluceptate (Eli Lilly & Co., Indianapolis, Ind.), ciprofloxacin (Miles Laboratories, Inc., West Haven, Conn.), sulfamethoxazole (Hoffmann-La Roche Inc., Nutley, N.J.), and three investigational macrolides—clarithromycin (Abbott Laboratories, North Chicago, Ill.), azithromycin (Pfizer Central Research, Groton, Conn.), and roxithromycin (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.). The various antimicrobial agents tested were supplied as powders and solubilized according to instructions from the manufacturer. No antibiotics or antifungal agents except those under investigation were included in the medium. Each concentration of each antimicrobial agent was tested in triplicate with each strain.

Susceptibility testing was performed in cell culture, using HeLa 229 cells in 96-well microdilution plates. Cells were pretreated with 30 μg of DEAE-dextran per ml for 10 min. Each well was inoculated with 0.1 ml of the test strain diluted to yield 10^3 to 10^4 inclusion-forming units per ml, centrifuged at 1,700 × *g* for 1 h, and incubated at 35°C for 1 h. Wells were then aspirated and overlaid with 0.2 ml of medium containing 1.0 μg of cycloheximide per ml and serial twofold dilutions of the test drug. After incubation at 35°C for 72 h, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the lipopolysaccharide genus antigen (Pathfinder, Kallestad, Chaska, Minn.). The MIC was the lowest antibiotic concentration at which no inclusions were seen. The minimal chlamydiacidal concentration (MCC) was determined by aspirating antibiotic-containing medium, washing wells twice with phosphate-buffered saline, and adding antibiotic-free medium. Cultures were frozen at −70°C, thawed, passed onto new cells, incubated for 72 h, and then fixed and stained as above. The MCC was the lowest antibiotic concentration which resulted in no inclusions after passage.

The MICs and MCCs for the three isolates are given in Table 1. There was no greater than a twofold dilution difference in MIC or MCC between isolates for a given drug. The macrolide antibiotics were more active than the other antibiotics tested, with clarithromycin the most active overall. In agreement with previous findings (3), all isolates tested were highly resistant to the sulfonamide that we tested.

Our results suggest a possible role for some of the newer macrolides and quinolones in the treatment of *C. pneumoniae* infections. Some of these agents may also be highly active against *Mycoplasma pneumoniae* (7), an important attribute since the clinical presentation may be quite similar. Timely definitive diagnosis is difficult for both *C. pneumoniae* and *M. pneumoniae*; therefore, empiric therapy should be active against both.

Few published data exist describing the clinical response of strain TWAR-associated pneumonia to antibiotic therapy. In one report every patient available for follow up had symptoms which persisted after therapy with erythromycin (2). The reasons for this poor response are unclear since the in vitro results presented here suggest that erythromycin and the other investigational macrolides tested have significant activity. These drugs, along with tetracycline, may have a role in the treatment of *C. pneumoniae* infections, although the optimum dose and duration of treatment are uncertain.

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TABLE 1. Activities of antibiotics against three strains of *C. pneumoniae* (*Chlamydia* sp. strain TWAR)

Antibiotic	TW-183		2043		2023	
	MIC (μg/ml)	MCC (μg/ml)	MIC (μg/ml)	MCC (μg/ml)	MIC (μg/ml)	MCC (μg/ml)
Erythromycin	0.06	0.125	0.125	0.125	0.06	0.125
Clarithromycin[a]	0.015	0.03	0.03	ND[b]	0.015	ND
Azithromycin	0.06	0.125	0.125	0.25	0.125	0.25
Roxithromycin	0.125	0.125	0.125	0.125	0.125	0.125
Tetracycline	0.125	0.06	0.125	0.125	0.125	0.06
Ciprofloxacin[a]	1.0	1.0	1.0	ND	1.0	ND
Sulfamethoxazole	>500	ND	>500	ND	>500	ND

[a] The MCCs for clarithromycin and ciprofloxacin were determined for TW-183 only.
[b] ND, Not done.

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