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MINIREVIEW

Antimicrobial Susceptibility and Therapy of Infections Caused by *Chlamydia pneumoniae*

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The first isolates of *Chlamydia pneumoniae* were serendipitously obtained during trachoma studies in the 1960s. TW-183 was isolated from the eye of a child with suspected trachoma in Taiwan, and IOL-207 was isolated from the eye of another child with trachoma in Teheran, Iran (15). Subsequently, serologic studies of an outbreak of mild pneumonia among schoolchildren in rural Finland in the late 1970s suggested that an organism related to TW-183 was the cause (15). Following the recovery of a similar isolate from the respiratory tract of a college student with pneumonia in Seattle, Grayston and colleagues (16) applied the designation TWAR after their first two isolates, TW-183 and AR-39. On the basis of inclusion morphology and staining characteristics in cell culture, TWAR was initially considered to be a *Chlamydia psittaci* strain. Subsequent analyses, however, have demonstrated that this organism is distinct from both *C. psittaci* and *Chlamydia trachomatis* and has been recognized as a third chlamydial species (15). Restriction endonuclease pattern analysis and nucleic acid hybridization studies suggest a high degree of genetic relatedness (>95%) among the *C. pneumoniae* isolates examined so far (15).

C. pneumoniae appears to be a primary human respiratory pathogen, and attempts to identify zoonotic reservoirs have been unsuccessful. The mode of transmission remains uncertain, but it is probably via infected respiratory secretions. Acquisition of infection by droplet aerosol has been described during a laboratory accident (23). *C. pneumoniae* can survive for several hours in laboratory-generated aerosols (39). Outbreaks of *C. pneumoniae* have occurred among members of enclosed populations such as military recruits (3, 15). The spread of infection has been also been documented among family members in the same household (15).

Serologic surveys have documented the rising prevalence of antibody to *C. pneumoniae* beginning in school-age children and reaching 30 to 45% by adolescence (15). The proportion of community-acquired pneumonias associated with *C. pneumoniae* infection has ranged from 6 to 19%, varying with geographic location and the age group examined (6, 14–16, 21, 40). Most infections with *C. pneumoniae* are probably mild or asymptomatic. Longitudinal serologic data obtained from military recruits in Finland suggest that only about 10% of infections results in clinically apparent pneumonia (15). The spectrum of disease associated with *C. pneumoniae* is expanding. Initial reports emphasized mild atypical pneumonia clinically resembling that associated with *Mycoplasma pneumoniae*. In one multicenter study of community-acquired pneumonia in children, *C. pneumoniae* was isolated from 16% of 26 children enrolled in the study, and 7 were coinfected with *M. pneumoniae* (21). These patients could not be differentiated clinically from those who were infected with *C. pneumoniae* or *M. pneumoniae* alone. Pharyngitis and bronchitis were seen in 1 and 5%, respectively, of college students with *C. pneumoniae* infection presenting with these complaints in Seattle (15, 40). *C. pneumoniae*-associated sinusitis has also been described both alone and in combination with lower respiratory tract infection. *C. pneumoniae* has also been isolated from the middle ear fluids of adults and children with otitis media with effusion (32). Infection with *C. pneumoniae* may be associated with reactive airway disease in children and new-onset asthma and asthmatic bronchitis in adults (12).

Another provocative and controversial development comes from data suggesting that *C. pneumoniae* may be an etiologic risk factor for coronary heart disease, including acute myocardial infarction. A recent report from the University of Washington has described the finding by electron microscopy of structures that appear to be *C. pneumoniae* elementary bodies in atheromatous plaques from coronary arteries obtained at autopsy (29).

As seen with genital infections caused by *C. trachomatis*, *C. pneumoniae* also appears to be capable of causing prolonged, often asymptomatic infections, but in the respiratory tract. Reports from Brooklyn, N.Y., Norway, and Sweden suggest that asymptomatic carriage may in fact be relatively common (3, 12, 23). Persistent nasopharyngeal infection with *C. pneumoniae* following acute respiratory infection has been documented in adults and children for periods of up to 11 months (12, 17).

Most of the studies mentioned above concentrated on the epidemiology and diagnosis of *C. pneumoniae* infections; treatment was almost an afterthought. Because most of the patients were identified retrospectively on the basis of serology, microbiologic efficacy could not be assessed. Given the broad range of illness associated with *C. pneumoniae* infection and its potential morbidity, attention has been turning to susceptibility testing and treatment studies.

SUSCEPTIBILITY TESTING OF *C. PNEUMONIAE*

The methods used for the susceptibility testing of *C. pneumoniae* have largely been adapted from those used for *C. trachomatis* and pose the same problems (11). The methods are not yet standardized, and results can be influenced by a number of variables including the type of tissue culture system, cell treatment, inoculum size, and timing of the addition of the antibiotic. Originally, it was found that *C. pneumoniae* grew

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poorly in egg and cell culture by using cells and conditions that were favorable for *C. trachomatis* (16). Kuo and Grayston (28) suggested that the use of HeLa cells pretreated with DEAE dextran was optimum for the culture and propagation of the organism. Even by these methods *C. pneumoniae* grew poorly, producing very small inclusions which were difficult to see by fluorescent-antibody staining (7). Isolation of the organism often required two to four serial passages (7). Subsequently, it was found that other cell lines, including HL and HEp-2 cells, were more sensitive to infection with *C. pneumoniae* (9, 33). Omission of pretreatment with DEAE dextran actually resulted in larger inclusions (33). As shown in Table 1, the majority of published studies of susceptibility testing of *C. pneumoniae* have used cycloheximide-treated HeLa or McCoy cells, most without pretreatment with DEAE dextran and with fluorescent-antibody staining with a genus-specific monoclonal antibody. Several studies have compared two or more cell lines, including HL and HEp-2 cells. In general, the results of MIC testing have been very consistent, irrespective of the cell line used.

All studies except one added overlay medium containing twofold dilutions of the test antimicrobial agent after the cell monolayers were inoculated (usually 30 min to 1 h after infection). Cooper et al. (10) also ran one set of experiments by adding the antibiotic to the growth medium before the cells were inoculated. The results shown in Table 1 are for the first method, the addition of antimicrobial agent after inoculation; the MICs obtained when the cells were infected after the addition of the antibiotics were the same for all of the antimicrobial agents tested except ciprofloxacin, the MICs of which were substantially lower (MIC 16 versus 2 μg/ml).

The majority of the investigators also determined minimum chlamydicidal concentrations (MCCs) by removing the antibiotic-containing medium from a duplicate plate and disrupting and passing the cells onto new monolayers. This is another direct adaptation of the methods used for the susceptibility testing of *C. trachomatis* (11). In most instances the MICs and MCCs of most of the antimicrobial agents tested were not significantly different.

The results of in vitro susceptibility testing indicate that *C. pneumoniae* has a pattern of susceptibility similar to that of *C. trachomatis* except for resistance to sulfonamides. Tetracyclines, macrolides, and quinolones are all active against *C. pneumoniae* in vitro. Few data are available on its susceptibility to beta-lactams. Kuo and Grayston (27) and Lipsky et al. (30) found that penicillin and ampicillin failed to suppress viability, i.e., the MIC, but were effective in inhibiting inclusion formation on passage (MCC). Although these results seem paradoxical, the data suggest that the inclusions seen in the presence of antibiotics at the MICs were not viable on passage.

One major reason for some of the variability in results may be the limited number of strains that have been available for testing. Of the 15 studies whose results are summarized in Table 1, 5 tested only one strain, 4 used TW-183, and 1 used IOL-207 (10, 13, 26, 32a, 42). One may argue that this is not significant because *C. pneumoniae* appears to be very homogeneous, with only one serovar identified so far. The 10 remaining studies tested three or more strains, and all included TW-183; 4 studies which tested three or four strains also used either AR39 and AR388 from Seattle or 2023 and 2043 from Brooklyn (2, 7, 38, 41). Only four studies used eight or more strains, including clinical isolates that were not passaged extensively. Only one study, that by Roblin et al. (35), examined a large number of recent clinical isolates with a wide geographic distribution. The MICs of clarithromycin ranged from 0.004 to 0.25 μg/ml; the MIC for 90% of the strains was 0.031 μg/ml. For only a few strains were MICs very low or very high. If only three to four strains are tested, one may not see this variation. The results of these studies reveal that there is strain-to-strain variation in susceptibility. As of this writing, there are only six strains of *C. pneumoniae* on deposit at the American Type Culture Collection (Rockville, Md.).

TREATMENT OF INFECTIONS CAUSED BY *C. PNEUMONIAE*

To date there have been few published data describing the response of *C. pneumoniae* infection to antimicrobial therapy. What is available are anecdotal reports or reports in abstract form. There are no studies that have systematically evaluated the microbiologic or clinical efficacy of antimicrobial therapy. Some of the available reports suggest that the regimens of erythromycin, tetracycline, and doxycycline that are effective against *C. trachomatis* are not effective against *C. pneumoniae*. In the original report by Grayston et al. (16) most of the patients were treated orally with 1 g of erythromycin per day for 5 to 10 days, which is one of the recommended regimens for *M. pneumoniae* infection. This appeared to be ineffective against *C. pneumoniae*, however, because many of these patients had continuing or recrudescing symptoms requiring additional treatment. Grayston et al. (16) then recommended either 2 g of tetracycline per day for 7 to 10 days or 1 g per day for 21 days. Because most of these patients were diagnosed serologically and follow-up cultures were not obtained, microbiologic efficacy could not be assessed. Subsequently, Hammerschlag et al. (17) reported that several patients with acute respiratory illness associated with positive *C. pneumoniae* cultures remained culture positive and symptomatic after receiving 2-week courses of erythromycin or 30-day courses of tetracycline or doxycycline.

A major problem in assessing the efficacy of antimicrobial treatment of *C. pneumoniae* infection is how one defines infection. Most investigators to date have relied on a serologic diagnosis obtained by the microimmunofluorescence (MIF) test. Grayston et al. (15) have proposed a set of criteria for the serologic diagnosis of *C. pneumoniae* infection and further suggest that this is more sensitive than culture. For acute infection, the patient should have a fourfold rise in the immunoglobulin G (IgG) titer or a single IgM titer of $\geq$1:16 or a single IgG titer of $\geq$1:512. Past or preexisting infection is defined as an IgG titers of $\geq$1:16 and $<$1:512.

However, it is important to realize that these criteria have some limitations. These criteria, especially those for use with a single sample, have not been correlated with the results of cultures in many studies. Use of serology with paired samples affords only a retrospective diagnosis and does not allow one to assess microbiologic efficacy. An example is a study by Lipsky et al. (30) on the use of ofloxacin for the treatment of pneumonia and bronchitis caused by *C. pneumoniae*. The patients described were enrolled in a treatment study comparing erythromycin with ofloxacin. Approximately 2 years later, the acute- and convalescent-phase sera were tested for *C. pneumoniae* antibody by MIF. Six patients were identified as having acute-phase *C. pneumoniae* antibody by the criteria given above. Four of these patients were treated with ofloxacin, and because they all improved clinically and because ofloxacin is active in vitro, with MICs of 1 to 2 μg/ml, it was assumed that ofloxacin was effective in treating these patients. However, subsequent studies in which culture as well as serology were performed have often found a poor correlation between the two. In a study of asymptomatic *C. pneumoniae* infection, Hyman et al. (23) found that results for single serum samples

TABLE 1. Comparison of antimicrobial susceptibility testing of *C. pneumoniae* by methods and results

Antibiotic	Reference	No. of strains	MIC (μg/ml)	MCC (μg/ml)	Cell line
Tetracycline	27	8	0.05–0.1	0.05–.01	HeLa
	13	1	1.0	ND[a]	McCoy
	30	3	0.05–0.1	0.05–0.1	HeLa
	10	1	0.5	>2	McCoy
	7	3	0.06–0.125	0.125	HeLa
	2	3	0.06–12	0.12	McCoy
	41	4	0.25–1.0	0.25–4	HeLa, HL
	42	1	0.5	1	McCoy
Doxycycline	19	11	0.06–0.25	0.125–0.25	HEp-2
	32a	1	0.05	0.5	McCoy
	13	1	0.25	ND	McCoy
	38	3	0.06–0.5	0.5–0.125	HeLa, McCoy, HL
	26	1	0.031	ND	HeLa
Minocycline	26	1	0.015	ND	HeLa
Erythromycin	27	8	0.08–0.1	0.05–0.1	HeLa
	7	3	0.06–0.125	0.125	HeLa
	30	3	0.01–0.05	0.01–0.05	HeLa
	13	1	0.06	ND	HeLa
	42	4	0.06–0.25	0.25–1	HeLa, HL
	32a	1	1	1.5	McCoy
	19	11	0.06–0.25	0.06–0.25	HEp-2
	26	1	0.25	ND	HeLa
	10	1	0.12	>1	McCoy
	35	49	0.016–0.125	0.016–0.25	HEp-2
Azithromycin	7	3	0.06–0.125	0.125–0.25	HeLa
	19	11	0.06–0.25	0.125–0.25	HEp-2
	13	1	1.0	ND	HeLa
	10	1	0.5	2	McCoy
	41	4	0.125–0.25	0.25–1	HeLa, HL
	2	3	0.12–0.25	0.25–0.5	McCoy
Clarithromycin	7	3	0.015–0.03	0.03	HeLa
	13	1	0.007	ND	McCoy
	10	1	0.035	0.03	McCoy
	19	11	0.004–0.03	0.008–0.03	HEp-2
	38	3	0.25	0.25	McCoy, HeLa
	35	49	0.004–0.25	0.004–0.25	HEp-2
	32a	1	0.25	1	McCoy
Roxyithromycin	3	3	0.125	0.125	HeLa
	13	1	0.25	ND	McCoy
Ciprofloxacin	7	3	1.0	1.0	HeLa
	13	1	2.0	ND	McCoy
	10	1	16	>16	McCoy
	18	10	0.25–4	0.25–8	HeLa, HEp-2
	26	1	1.0	ND	HeLa
	42	1	2	2	McCoy
Ofloxacin	13	1	1.0	ND	McCoy
	30	3	1.0–2.0	1–2	HeLa
	18	10	0.5–2	0.5–2	HeLa, HEp-2
	26	1	0.5	ND	HeLa
	34	12	0.5–2	0.5–2	HEp-2
Levofloxacin	19	11	0.125–0.5	0.125–0.25	HEp-2
Fleroxacin	13	1	2.0	ND	McCoy
	18	6	2–8	2–8	HeLa, HEp-2
Lomefloxacin	13	1	4	ND	McCoy
Temafloxacin	13	1	0.5	ND	McCoy
	18	10	0.125–1	0.125–2	HeLa, HEp-2
	26	1	0.125	ND	HeLa

Continued on following page

TABLE 1—*Continued*

Antibiotic	Reference	No. of strains	MIC (μg/ml)	MCC (μg/ml)	Cell line
Sparfloxacin	10	1	0.5	>2	McCoy
	18	6	0.06–0.25	0.06–0.25	HeLa, HEp-2
	26	1	0.06	ND	McCoy
	34	12	0.06–0.25	0.06–0.25	HEp-2
OPC17116	26	1	0.063	ND	McCoy
	34	12	0.25–0.5	0.25–0.5	HEp-2
	42	1	0.06	0.03	McCoy
Ampicillin	27	8	>100	0.8–1.6	HeLa
	14	3	>100	0.8–1.6	HeLa
Penicillin	22	8	>100	0.1–0.2	HeLa
	10	3	>100	0.1–0.2	HeLa
Sulfisoxazole	27	8	>400	≥400	HeLa
	10	3	>400	≥400	HeLa
Sulfamethoxazole	7	3	>500	ND	HeLa

[a] ND, not determined.

from 12 (17%) of 72 healthy, culture-negative adult health care workers were suggestive of acute infection (IgM titer ≥ 1:16 or IgG titer ≥ 1:512). Similar results were reported by Kern et al. (25) among healthy firemen in Rhode Island; serologic evidence of recent *C. pneumoniae* infection was present in 13% of them. In contrast, of 22 *C. pneumoniae* culture-positive adult patients reported in three separate studies, only 3 fulfilled the conventional serologic criteria for recent infection (3, 6, 22). Most of these patients had pneumonia or bronchitis; two were asymptomatically infected after a laboratory accident. Two studies which identified 54 children with community-acquired pneumonia and asthma who were culture positive for *C. pneumoniae* found that only 12 (22%) had serologic evidence of acute infection and 40 (74%) had no detectable antibody by MIF even after 2 or more months of follow-up (12, 21).

Recently, the U.S. Food and Drug Administration and the Infectious Disease Society of America drafted a set of general guidelines for the evaluation of new anti-infective agents in the treatment of various infectious diseases. In the section on respiratory tract infections, under the clinical definition of the disease, it was stated that isolation by culture is not required for the diagnosis of pneumonia caused by *C. pneumoniae* (8). However, it is clear from the preceding data that serology by the MIF test may not accurately reflect the culture status of the patient. This situation appears to be analogous in many ways to that for genital infection with *C. trachomatis*; no one would even consider evaluating antimicrobial efficacy by serology. However, the only treatment studies of pneumonia caused by *C. pneumoniae* that have been published in journals so far have relied entirely on diagnosis by serology (1, 37).

In three studies of the treatment of *C. pneumoniae* infection, cultures were performed; one has been published recently, one was presented as an abstract, and one is in press. Two were studies of pediatric populations. One was a multicenter study (4) comparing erythromycin suspension, 40 mg/kg of body weight per day for 10 days, with clarithromycin suspension, 15 mg/kg/day for 10 days, in children 3 to 12 years of age with radiographically proven pneumonia. Of 33 evaluable culture-positive patients, the organism was eradicated from the nasopharynxes of 12 of 14 (86%) of the children who were treated with erythromycin and 15 of 19 (79%) of the children who received clarithromycin. However, all of the children with persistent infection improved clinically, with complete resolution of the chest x rays. In another study (12), 12 children (ages 5 to 15 years) with asthma who were culture positive for *C. pneumoniae* were treated with erythromycin suspension, 50 mg/kg/day for 2 weeks, or clarithromycin, 15 mg/kg/day for 10 days. All six children who received erythromycin were culture negative after treatment. One of six children who was treated with clarithromycin was culture positive after treatment, remained culture positive after a second course of clarithromycin, and finally became culture negative after 3 weeks of erythromycin therapy. Overall, nine (75%) of these children demonstrated impressive improvements in their reactive airway disease, with eradication of the organism.

In the study involving adults (20), *C. pneumoniae* infection was identified by culture in 16 of 62 (26%) adults (ages, 16 to 77 years) with acute bronchitis or pneumonia. All 16 culture-positive patients, 15 with bronchitis and 1 with pneumonia, were treated with azithromycin at a 1.5-g total oral dose over 5 days. *C. pneumoniae* was successfully eradicated from the nasopharynxes of 12 (75%) of the patients when they were seen 4 to 6 weeks after treatment. However, all four patients with persistent infections improved clinically, including two who were coinfected with *M. pneumoniae*. *M. pneumoniae* was eradicated at the posttreatment visits.

It is unclear why antibiotic regimens that are very effective against infections caused by *C. trachomatis* appear to be less so against infections caused by *C. pneumoniae*. This is especially puzzling because the MICs and MCCs of most of these agents are essentially the same for both organisms (11). It is also evident that the results of in vitro susceptibility testing may not predict microbiologic efficacy in vivo. Clarithromycin is one of the most active antibiotics against *C. pneumoniae* in vitro and, in addition, has excellent tissue and intracellular penetrations (36). Azithromycin has activity similar to that of erythromycin against *C. pneumoniae*, but it is widely distributed in tissue and has superior pharmacokinetics (36). These characteristics would suggest that these drugs would be superior to erythromycin for the treatment of *C. pneumoniae* infection, yet these preliminary studies found the efficacies of these drugs to be equivalent. However, the numbers of subjects in the studies reported so far are so few that it precludes determination of any statistically significant difference among the regimens.

Another possibility for treatment failure may be the development of antibiotic resistance. Although the relative resistance of *C. trachomatis* to erythromycin and doxycycline has been reported, the relationship to treatment failure is unclear (24, 31). The doxycycline resistance appeared to be related to an inoculum effect, which has not yet been described for *C. pneumoniae*. Forty-nine isolates from 35 of the children with pneumonia, including 8 who were persistently positive, were retrieved and tested for their susceptibilities to erythromycin and clarithromycin in vitro; all were found to be uniformly susceptible to both antibiotics, and the MICs and MCCs did not change during therapy (35). These data suggest that the persistence of infection is not secondary to the development of antibiotic resistance. Persistence may be related to the levels of drug in tissue and the slower cellular turnover rate in the respiratory tract.

The results of these preliminary studies emphasize the need for a specific microbiologic diagnosis. Grayston et al. (16) and other investigators (21) have remarked that patients with *C. pneumoniae* infection could not be differentiated clinically from patients with other infections, especially *M. pneumoniae*. A number of patients in the pediatric pneumonia study and the adult bronchitis-pneumonia study were found to be coinfected with *C. pneumoniae* and *M. pneumoniae* (20, 21). One could not differentiate these patients on the basis of history, cough, fever, leukocyte count, and appearance of chest x ray from those patients infected with only one pathogen. Considering the significant overlap in clinical presentation, it would appear that the presumptive therapy of community-acquired lower respiratory tract infection should be directed at *C. pneumoniae* and *M. pneumoniae*. On the basis of the currently available data, it appears that, for adults, 2 to 3 weeks of doxycycline or erythromycin (at 2 g per day) or azithromycin at 1.5 g over 5 days are equivalent. For children, either erythromycin or clarithromycin suspension for 10 days to 2 weeks is required. The choice of regimen will depend on patient compliance, tolerance, and cost. Prospective studies by culture methods are needed to determine antimicrobial efficacy and the best therapeutic regimen for the treatment of respiratory infections caused by *C. pneumoniae*.

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