Potential for Antimicrobial Resistance in *Chlamydia pneumoniae*

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Antimicrobial resistance has not yet been described in wild type *Chlamydia pneumoniae* isolates, nor has selective emergence of resistance in the laboratory after exposure to subinhibitory concentrations of antibiotic. However, few clinical isolates have been tested for resistance, especially strains with resistance phenotypes (i.e., those associated with clinical failure or persistence). More widespread testing of such strains is needed. Further understanding of antimicrobial resistance in chlamydiae would benefit from the development of standardized methods. Further, more physiologic testing methodologies that more closely mimic the chronic intracellular infection usually being treated in vivo would be of value. Animal models demonstrate persistence of *C. pneumoniae* after antimicrobial therapy and could be used to better define the clinical correlates of in vitro testing.

Increasing resistance to antimicrobials has been of considerable concern with many bacterial infections but has not as yet been frequently observed with *Chlamydia* organisms. One potential explanation for the lack of resistance by chlamydiae may be their unique life cycle; chlamydiae gene replication occurs exclusively within the intracellular inclusion it establishes in infected cells. Thus, replication occurs in relative isolation where acquisition of antibiotic resistance genes from other organisms would be difficult. However, a variety of factors may contribute to underrecognition of antimicrobial resistance in chlamydiae. First, few infections are actually documented by culture with most being treated empirically in patients presenting with chlamydiae-associated syndromes (i.e., nongonococcal urethritis or pelvic inflammatory disease for *Chlamydia trachomatis* or community-acquired pneumonia or upper respiratory infection for *Chlamydia pneumoniae*). In addition, non-culture tests such as polymerase chain reaction or ligase chain reaction (LCR) are increasingly being used diagnostically, and these tests do not permit susceptibility testing. Even in patients who have microbiologically documented infections, test-of-cure cultures are rarely done and have not been recommended due to the apparent high cure rates achieved in treatment of chlamydial infections. Other factors that may contribute to our potential failure to recognize antimicrobial resistance include the relative insensitivity of current culture systems, which may fail to culture low inoculum infections in the posttreatment setting and the time consuming and expensive methods that are currently used for assessment of chlamydial antimicrobial resistance. Finally, there has been a tendency to attribute recurrent chlamydial infection to factors other than antimicrobial resistance, including reinfection, poor compliance with medication, or persistence.

Despite the current lack of evidence for frequent antimicrobial resistance in chlamydiae, there are good reasons to suspect that resistance would likely occur. The infection is often chronic, asymptomatic, and persistent, and chlamydiae-infected patients may thus be exposed to intermittent, incomplete, or recurrent antimicrobial treatment episodes [1], factors that promote resistance in other chronic intracellular infections such as mycobacterial infections. Intermittent mass therapy as has been used for trachoma might also exert selective pressure for resistance [2]. Thus, for these reasons, concern regarding antimicrobial resistance in chlamydiae is clearly warranted.

Current Methods for Assessment of Antimicrobial Resistance in Chlamydiae

The methods currently utilized for assessment of antimicrobial resistance in chlamydiae are less than ideal for a number of reasons. First, the systems generally are “unphysiologic” in a number of ways. In natural infections, chlamydiae are usually only exposed to antimicrobials long after an intracellular infection has been well established, an inflammatory response induced, and often after chronicity and infection of several cell types has occurred. In contrast, the in vitro systems used for testing antimicrobial resistance in chlamydiae could be characterized as models of “hyperacute” infections in that the antimicrobials are added soon (sometimes simultaneously with) the infectious agent rather than after a chronic, persistent infection is present [3].

Kutlin et al. [4] reported one effort to establish persistent infection with a continuous infection model of *C. pneumoniae* in which HEp-2 cells were persistently infected with the CM-1 or TW-183 strain and cells were cultivated without cyclohexamide or centrifugation [4]. With this system, cycles of host cell lysis, detachment, and regrowth were observed, and per-
sisting infection was observed for more than 1 year. In this system, azithromycin and ofloxacin reduced but did not eliminate *C. pneumoniae* infection. Another more physiologic system utilized epithelial cells grown in polarized monolayers for susceptibility testing [5]. Further development and use of such systems for susceptibility testing of chlamydiae would be useful, including approaches that would assess the effects of an antimicrobial on different parts of the chlamydial life cycle.

Recent data also demonstrate the secretion of cytokines by epithelial and inflammatory cells infected with chlamydiae [6]. It is possible that these molecules may influence the subsequent microbiocidal effects observed in a system being used to assess antimicrobial susceptibility. Further assessment of the potential effects of cytokines upon antimicrobial susceptibility testing of chlamydiae is needed.

In addition to the conceptual concerns outlined above, there are many practical methodologic issues related to testing for antimicrobial resistance in chlamydiae. The methods currently in use are unstandardized and vary considerably from laboratory to laboratory [3, 7]. The effects of many important variables in the system have been incompletely studied, including the inoculum size, the cell type used, the interval between establishment of infection and addition of antibiotic, the effects of different media, and the timing of antibiotic removal. Antibiotics may differ in their specific effects on different parts of the chlamydial life cycle, an area deserving of further study. Also of great importance is establishment of the most meaningful outcome: MIC, MBC, MIC\_90, and others and whether these outcome measures determined in vitro actually predict clinical outcomes in experimental animals or humans.

### Clinical Chlamydia Isolates with Antimicrobial Resistance

Relatively few clinical *Chlamydia* isolates with antimicrobial resistance have been described. In 1990, Jones et al. [10] identified *C. trachomatis* isolates described as resistant to tetracycline, doxycycline, erythromycin, and clindamycin but susceptible to ofloxacin and ciprofloxacin. Resistance was only apparent when a high inoculum was used in the cell culture system and only about 1% of the inoculum appeared to be resistant. Further, the strains did not demonstrate a stable resistance phenotype upon serial passage, and there was no apparent clinical correlation between resistance and treatment failure. More recently, Lefevre et al. [11] reported a single *C. trachomatis* strain as tetracycline resistant. This isolate was from a patient who was persistently symptomatic after doxycycline therapy. The isolate’s MIC and MBC for tetracycline were >64 mg/mL and, as reported by Jones et al. [10], fewer than 1% of the organisms were resistant. The patient was successfully treated with pristinamycin, to which the organism was susceptible (MIC/MBC, 0.25/0.5 mg/mL).

Most recently, Black et al. [12] reported 3 patients who appeared to have strains of *C. trachomatis* that exhibited multiple antimicrobial resistance in the setting of persistent clinically apparent infection and persistent positive LCRs or cultures. One pregnant woman had persistent positive LCRs despite treatment with several antimicrobials, including erythromycin, amoxicillin, and azithromycin. The strain exhibited an MCC of >4.0 mg/mL to azithromycin, ofloxacin, and doxycycline. Two other patients (a husband and wife) also had infection with a strain shown to be identical by OMP-1 genotype with a resistance profile similar to that described for the first patient.

*C. pneumoniae* clinical isolates with evidence of resistance to antimicrobials have not yet been described. However, Hammerschlag et al. [13] described 5 patients with culture-positive *C. pneumoniae* respiratory infections who had multiple positive cultures over several months despite appropriate antibiotic therapy. The strains were not tested for antimicrobial resistance, but the data suggest that *C. pneumoniae* may persist despite antibiotic therapy in some patients, with or without symptoms. In another study, Robin et al. [14] tested the MICs of 55 *C. pneumoniae* isolates from 46 patients with respiratory infection. The MIC\_90 was 0.5 mg/mL for these strains. In 7 patients, cultures were positive after therapy and in 2 of these patients, the posttreatment MIC had increased four-fold; however, the patients had improved clinically.

To date, few *C. pneumoniae* isolates from nonrespiratory tract sites have been tested for antimicrobial resistance. Gieffers et al. [15] tested 5 *C. pneumoniae* isolates from vascular tissue in patients with atherosclerotic cardiovascular disease and found the strains were no different than respiratory strains in terms of antimicrobial susceptibility (i.e., susceptible to azithromycin, erythromycin, ofloxacin, doxycycline, and rifampin). Also of interest are the studies of Freidank et al. [16], who tested 12
C. pneumoniae strains (6 clinical and 6 reference strains) for evidence of synergy in vitro when antimicrobial combinations were used. The MIC/MBC of these strains were typical of other reported strains, but many of the combinations tested demonstrated synergy, including azithromycin plus ofloxacin, azithromycin plus doxycycline, azithromycin plus rifampin, azithromycin and rifampin and doxycycline, and azithromycin, rifampin, and ofloxacin.

**Experimental Animal Models to Assess Cure of C. trachomatis and C. pneumoniae Infections**

Experimental animal models have been used to evaluate the therapeutic efficacy of antimicrobials against “susceptible” C. trachomatis and C. pneumoniae strains. With C. trachomatis, persistence of live organisms and of chlamydial DNA following antimicrobial therapy have been demonstrated in experimental infections in the primate model [17]. The strains used were considered susceptible, and the antimicrobial resistance profiles after therapy was not tested to evaluate emergence of resistance during treatment. In an older study with the mouse pneumonia strain, Loosli et al. [18] observed the development of resistance to sulfadiazine in a pulmonary infection model in mice. With C. pneumoniae infection in the mouse model, the AR-39 strain of C. pneumoniae was most effectively eradicated from lung tissue by the combination regimen of azithromycin and rifampin; azithromycin alone was less effective. With this model system, animals that were culture negative after antibiotic treatment could be made culture positive again following treatment with corticosteroids, suggesting reactivation of persisting infection with immunosuppression [19]. As yet, no attempts have been reported of infecting animals with C. trachomatis or C. pneumoniae strains that are considered resistant to antimicrobials.

**Conclusions**

Antimicrobial resistance has not yet been described in wild type C. pneumoniae isolates nor has there been selective emergence of resistant strains in the laboratory after exposure to subinhibitory levels of antibiotic. However, few clinical isolates have been tested for resistance, especially strains from resistance phenotypes (i.e., strains associated with clinical failure after antibiotic therapy, with persistence, or with complications of C. pneumoniae infection). More widespread testing of such strains with resistance phenotypes would be useful. Animal models demonstrate persistence of C. pneumoniae after antimicrobial therapy and should be used to evaluate potentially resistant strains and to define the clinical correlates of in vitro testing.

With C. trachomatis infections, resistant clinical isolates associated with treatment failure have been reported. Resistance to antimicrobials in C. trachomatis can also be induced in the laboratory with exposure to subinhibitory levels of antimicrobials. Many C. trachomatis strains considered resistant seem to exhibit heterotypic resistance in which only 1% of the organisms demonstrate resistance. Further understanding of antimicrobial resistance in chlamydiae would benefit from the development of more standardized and more physiologic methods. Experiments better defining the optimal inoculum, the cell type infected, the antimicrobial exposure time, and most appropriate end points would be especially useful. An experimental system more closely mimicking chronic intracellular infection is likely to produce results that better approximate in vivo conditions. Correlation between specific end points in vitro (i.e., MBC, MIC<sub>90</sub>) and cure in animals would facilitate a better understanding of how well in vitro testing actually predicts clinical outcome. Finally, testing of a greater number of strains for resistance, especially strains from resistant phenotypes, would provide a better estimate of the prevalence of resistance and its clinical correlates in chlamydiae.

**References**


18. Loosli CG, Grayston JT, Alexander ER. Experimental airborne mouse pneumonitis virus infection in mice. III. The development of a strain of MPV resistant to sulfadiazine and related compounds. Antibiotics Annual (Medical Encyclopedia) 1954:490.