In Vitro Susceptibilities of *Chlamydia pneumoniae* Strains Recovered from Atherosclerotic Coronary Arteries

JENS GIEFFERS,* WERNER SOLBACH, AND MATTHIAS MAASS

Institute of Medical Microbiology and Hygiene, Medical University of Lübeck, D-23538 Lübeck, Germany

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*Corresponding author. Mailing address: Institute of Medical Microbiology and Hygiene, Medical University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany. Phone: 49 (451) 500-2818. Fax: 49 (451) 500-2808. E-mail: gieffers@hygiene.mu-luebeck.de.

**Chlamydia pneumoniae** strains have been recovered from arteriosclerotic coronary arteries, but their antibiotic susceptibility profiles have not yet been examined. We report in vitro susceptibility data for five cardiovascular *C. pneumoniae* isolates. These strains did not differ significantly from respiratory strains in their patterns of susceptibility to azithromycin, erythromycin, roxithromycin, ofloxacin, doxycycline, rifampin, and penicillin G. Roxithromycin was the most active macrolide, and rifampin was the most effective drug overall.

_Chlamydia pneumoniae_, a common cause of respiratory diseases, has been established as a new species of the genus _Chlamydia_. It appears that virtually all humans are infected with _C. pneumoniae_ at some time during their lives (3). Shortly after the discovery of the obligate intracellular pathogen, seroepidemiological studies reported an association between coronary artery disease and prior _C. pneumoniae_ infection (2, 12, 13, 18). This statistical relation was corroborated by detection of chlamydial structures in various arterial vessels (1, 10, 15). Though a causal role of _C. pneumoniae_ in the development of atherosclerosis has not been established, pilot studies of antibacterial treatment in patients with coronary artery disease have been performed already (4, 5) and further large-scale trials are under way. Since vascular _C. pneumoniae_ isolates have not been available until very recently (7, 11, 16), drug selection had to be based upon susceptibility data derived exclusively with respiratory strains. Hypothetically, vascular strains might represent a chlamydial subpopulation with susceptibility profiles differing from those of respiratory strains. In the present study, susceptibility profiles of _C. pneumoniae_ strains that have been directly recovered from arteriosclerotic coronary arteries are reported for the first time; the data may contribute to the development of an eradication therapy for endovascular chlamydiae.

Five cardiovascular strains (designated CV-1 and CV-3 to CV-6) and two respiratory reference strains (MUL-1 and CWL-029 [ATCC VR 1310]) were compared for antibiotic susceptibility. The cardiovascular strains were recently recovered from coronary arteries of patients undergoing coronary bypass surgery in Lübeck, Germany, as described previously (11). The MUL-1 strain is a local clinical isolate from a patient suffering from severe respiratory illness (14). All strains were continuously grown on HEp-2 cells. The antimicrobial agents were ofloxacin, erythromycin, roxithromycin, doxycycline, rifampin, penicillin G (all from Sigma, Deisenhofen, Germany), and azithromycin (Mack, Illertissen, Germany). They were supplied as powders and solubilized according to the manufacturers’ instructions. Susceptibility testing was performed on HEp-2 monolayers grown in 24-well plates. Culture conditions were as described previously (14). After the cell sheet reached confluence, the growth medium—consisting of Eagle’s minimal essential medium (Gibco, Eggenstein, Germany), 10% fetal bovine serum (Biochrom, Berlin, Germany), and nonessential amino acids (Gibco)—was replaced by serum-free Eagle’s minimal essential medium supplemented with 1 μg of cycloheximide (Sigma) per ml and one of the studied antibiotics in twofold dilutions. No additional antimicrobial agents were used in the culture system. Each well was inoculated with 0.5 ml of the test strain diluted to yield 10⁷ inclusion-forming units (IFU) per ml and centrifuged at 3,000 × g for 45 min. After incubation at 35°C and 5% CO₂ for 72 h, the monolayers were methanol fixed and stained with a chlamydia-specific monoclonal fluorescein isothiocyanate-coupled antibody (Dako, Ely, England). The MIC was defined as the lowest concentration at which no IFU were observed. In accordance with established protocols (6), the minimal chlamydial concentration (MCC) was subsequently determined by removing the drug-containing medium, washing the wells with phosphate-buffered saline, and adding fresh medium without antibiotic supplementation. The infected monolayer was then disrupted by the addition of glass beads and vigorous shaking of the plates for 2 h. The disrupted cells were passed on fresh monolayers, again incubated for 72 h, and stained as described above. The MCC was the lowest drug concentration that inhibited the production of IFU in the antibiotic-free passage. All titrations were carried out in triplicate.

The results of MIC and MCC testing are summarized in Table 1. The variation of a triple titration was within one dilutional step. All strains were completely susceptible to the applied drugs with the exception of penicillin G. Differences between respiratory and cardiovascular strains were minimal: there was no dilutional difference greater than twofold between the tested strains. For a given drug, the MIC and MCC were equal or varied by one dilutional step. On a weight and a molar basis, the activities of macrolides and doxycycline were distinctly higher than the activity of ofloxacin. Roxithromycin was the most active macrolide. Rifampin was the most effective antichlamydial drug, with MICs and MCCs of 0.005 μg/ml. In an additional experiment, two noninhibitory concentrations of rifampin (0.0025 and 0.0001 μg/ml) were applied to the wells containing dilutions of the antibiotics. This approach did not result in a reduction of the pertinent MICs (data not shown). Penicillin G did not completely inhibit inclusion formation at the maximum concentration used. However, an antimicrobial influence resulting in morphologically aberrant IFU and
weaker staining was observed at penicillin G concentrations of >0.5 μg/ml. This is the first study to investigate the antibiotic susceptibility of cardiovascular strains of *C. pneumoniae*. Apart from the five strains used here (11), only two other isolates have been recovered from atheromatous plaques (7, 16), and their susceptibility patterns have not yet been reported. In our study, no difference was observed among the vascular isolates or between the isolates and the respiratory strains. Apparently, vascular isolates of *C. pneumoniae* do not have a distinctive susceptibility profile. This confirms the finding that the *C. pneumoniae* isolates obtained so far are very homogeneous in their genetic background, protein profile, and immunogenicity.

This lack of discriminatory characteristics among the respiratory strains has resulted in the preliminary designation of only one serovar, TWAR, within the species (3). However, since a genetic typing system has not yet been established for *C. pneumoniae*, the presence of a genotype with a distinct vascular background, protein profile, and immunogenicity. Their genetic background, protein profile, and immunogenicity. Their genetic background, protein profile, and immunogenicity. Their genetic background, protein profile, and immunogenicity. Their genetic background, protein profile, and immunogenicity. Their genetic background, protein profile, and immunogenicity. Their genetic background, protein profile, and immunogenicity. Their genetic background, protein profile, and immunogenicity.

On a weight and a molar basis, rifampin was the most active antichlamydial drug in this investigation. Little is known about its efficacy against *C. pneumoniae*, but rifampin is the most effective antibiotic against *Chlamydia trachomatis* and shows a clinical efficacy similar to that of tetracycline (19). In our study, combinations of erythromycin or doxycycline with rifampin were neither synergistic nor antagonistic (data not shown), which is in accordance with previous studies on *C. trachomatis* (8).

All MICs and MCCs were, without exception, within the range known for respiratory isolates, even though this range is due to a variation of procedural details (6). Morphologically aberrant inclusions bodies produced in the presence of antibiotics make the determination of breakpoints difficult. MICs exceeding MCCs, as observed especially for penicillin G, appear somewhat paradoxical, but this phenomenon was observed in earlier studies, too (9). Apparently, inclusions can be produced in the presence of antibiotic concentrations near the MIC but then lose their viability because they are not able to replicate in subcultures.

A recent study reported a poor correlation between *C. pneumoniae* in vitro susceptibility data and clinical treatment results (17). Therefore, in vitro susceptibility data may not necessarily reflect clinical efficacy. However, these data demonstrate that endovascular chlamydiae are principally susceptible to antimicrobial treatment. Two recent clinical studies that used roxithromycin or azithromycin in patients with coronary artery disease have reported a significant reduction in the frequency of cardiovascular events (5) and a decrease in *C. pneumoniae* antibody titers after therapy (4). These data are still preliminary and at this time by no means warrant the use of antimicrobial therapy to treat coronary artery disease. Whether chlamydiae actually have an etiological role in the development of coronary atherosclerosis is not known; a mere colonization of preexisting plaques cannot be excluded. However, the in vitro results reported in the present study may provide a basis for those observations and for future evaluation of the potential clinical benefit of an eradication therapy for endovascular chlamydiae. Since a broad range of antibiotics has distinct activity against *C. pneumoniae*, the choice of antimicrobial agents may be more influenced by pharmacokinetic parameters and the duration and cost of the antibiotic therapy than by differences in the in vitro efficacies of the antibiotics.

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### REFERENCES


### TABLE 1. Activities of antibiotics against cardiovascular strains (CV-1 and CV-3 to CV-6) and respiratory strains (CWL-029 and MUL-1) of *C. pneumoniae*

<table>
<thead>
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<th>Antibiotic</th>
<th>CWL MIC</th>
<th>CWL MCC</th>
<th>MUL-1 MIC</th>
<th>MUL-1 MCC</th>
<th>CV-1 MIC</th>
<th>CV-1 MCC</th>
<th>CV-3 MIC</th>
<th>CV-3 MCC</th>
<th>CV-4 MIC</th>
<th>CV-4 MCC</th>
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<th>CV-5 MCC</th>
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* ND, not done.