In Vitro Activity of Clavulanic Acid, Amoxicillin, and Ticarcillin against *Chlamydia trachomatis*

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In vitro, growth of *Chlamydia trachomatis* was not entirely eliminated by 960 μg of ticarcillin per ml, 64 μg of amoxicillin per ml, 32 μg of clavulanic acid per ml, a combination of ticarcillin (480 μg/ml) and clavulanic acid (32 μg/ml), and a combination of amoxicillin (32 μg/ml) and clavulanic acid (8 μg/ml). However, a ≥99% decrease in the number of inclusions was obtained at concentrations readily attainable in serum.

Penicillins have partial activity against *Chlamydia trachomatis* both in vitro and in vivo (2–7, 9, 16). For most antimicrobial agents, MICs are well defined and the number of inclusions in cell cultures declines dramatically from numbers near those in controls without antimicrobial agents to nil over only a few dilutions (6, 10, 14, 15). In contrast, with penicillins there are occasional inclusions over a wide range of concentrations (6, 10, 14, 15). This probably accounts for the large discrepancies in published reports of MICs of penicillins (4, 6, 9, 10, 13–16). In vivo, single-dose penicillin treatment does not usually eradicate *C. trachomatis* (7, 10), but multiple-dose penicillin treatment often results in negative follow-up cultures for weeks or longer (2, 5, 7, 12). Because of this activity, penicillins have been considered for use in the treatment of uncomplicated chlamydial infections in pregnancy (2). Clavulanic acid, in combination with amoxicillin or ticarcillin, has been used in the treatment of genital syndromes or infections such as the frequency-dysuria syndrome and pelvic inflammatory disease (1, 8) that are sometimes caused by *C. trachomatis* (11, 17). Consequently, it is important to know whether the addition of clavulanic acid to amoxicillin or ticarcillin enhances in vitro activity against *C. trachomatis*. In this study, the activities of amoxicillin, ticarcillin, and clavulanic acid alone, clavulanic acid in combination with amoxicillin, and clavulanic acid in combination with ticarcillin were evaluated in vitro against *C. trachomatis* in cell cultures.

Twenty-one strains of *C. trachomatis* were used, including three typed strains (B, DE, and FG) initially isolated from men with nongonococcal urethritis in Seattle, Wash., one LGV strain kindly provided by Julius Schachter, and 17 genital isolates from men and women in Vancouver or Edmonton, Alberta, Canada. Isolates were passed in cycloheximide-treated McCoy cells and stored at −70°C when not in use. All strains were in passage 7 to 10 at the time of use.

Antimicrobial agents were reconstituted and diluted with 0.4 M sucrose phosphate the day of use (6, 15). The final concentrations evaluated for ticarcillin were 960, 480, 30, 7.5, 1.875, and 0.469 μg/ml; for amoxicillin they were 64, 16, 4, 1, and 0.25 μg/ml; for clavulanic acid they were 32, 16, 4, 1, and 0.25 μg/ml; for the combination of ticarcillin and clavulanic acid in a 15:1 ratio they were 480 and 32, 30 and 2, 7.5 and 0.5, 1.875 and 0.125, and 0.469 and 0.031 μg/ml; and for the combination of amoxicillin with clavulanic acid in a 4:1 ratio they were 32 and 8, 8 and 2, 2 and 0.5, 0.5 and 0.125, and 0.125 and 0.031 μg/ml.

Experiments were performed in triplicate in 1-gram (ca. 1.8-g) vials by using a methodology previously described by me and others (6, 15). Briefly summarized, *C. trachomatis* inocula yielding 20 to 30 inclusion forming units per 400 power field were centrifuged onto monolayers at 2,300 × g for 1 h, the antimicrobial agents were then added, and the cultures were incubated. In MIC experiments to determine the minimal amount of antimicrobial agent required to prevent development of *C. trachomatis* inclusions, the vials were incubated for 72 h and then stained with iode. For MBC experiments to determine the minimal amount of antimicrobial agent that resulted in no inclusion formation when infected monolayers exposed to antimicrobials agents were passed to new monolayers without antimicrobial agents, the monolayers were incubated for 48 h, rinsed twice with 0.04 M sucrose phosphate, and then overlaid with 0.04 M sucrose phosphate and frozen at −70°C. They were then thawed and inoculated onto fresh cells, incubated for 72 h, and stained with iode. The MIC and MBC are the concentrations at which no inclusions were seen in triplicate vials.

The results are shown in Table 1. For all antimicrobial agents or combinations of antimicrobial agents, the median MIC for 10 isolates exceeded the highest concentration of antimicrobial agents studied. For one strain with amoxicillin, three strains with clavulanic acid, one strain with ticarcillin-clavulanic acid, and three strains with amoxicillin-clavulanic acid, no inclusions were seen at the highest concentration of antimicrobial agent. At much lower concentrations, there was a very significant diminution in the number of detectable inclusions. The concentration of antimicrobial agent at which there was a ≥99% decrease in the number of inclusions in MIC experiments compared with simultaneously run controls without antimicrobial agents is also shown in Table 1. The median concentrations required were all in the range that is readily attainable in serum. For all but clavulanic acid the concentrations producing a ≥99% decrease in inclusion formation were consistent among strains, and the median (Table 1) was the actual endpoint in ≥71% of strains. For clavulanic acid by itself, results were less consistent, and the concentration required to produce a ≥99% decrease was ≤0.25 μg/ml for 3 strains, 1.0 μg/ml for 10 strains, 4 μg/ml for 7 strains, and 16 μg/ml for 1 strain.
MIC and MBC procedures were performed on six strains. In almost all situations the results were identical. That is, inclusions were detected at the highest concentration of antimicrobial agent tested.

Inclusions at high concentrations were highly aberrant in appearance but were detectable. Aberrant inclusions with penicillins have long been known (13). Even in the presence of 100 μg of penicillin per ml, C. trachomatis-specified protein synthesis continues (13). The difficulty in detection has resulted in considerable disagreement in reported MICs. Thus, for antimicrobial agents such as ticarcillin, ampicillin, and similar compounds and for ticarcillin and related compounds, some groups have reported very high MICs (4, 6, 10, 15; R. Suchland, L. D. Cles, and W. E. Stamm, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 455, 1984), some have reported intermediate MICs (D. H. Martin, J. G. Pastorek, and S. Faro, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1124, 1984), and still others have reported low MICs (9, 12, 14, 16). Results with MBC techniques support the validity of the failure of these antimicrobial agents to kill C. trachomatis in vitro (6, 14–16). Furthermore, use of fluorescein-conjugated monoclonal antibody to C. trachomatis shows high MICs for penicillin (Suchland et al., 24th ICAAC). This technique circumvents potential difficulties in identifying inclusions by the iodine staining method used in this study. Thus, despite the discrepancies in the literature, it seems unlikely that penicillins can totally stop in vitro replication of C. trachomatis in concentrations that are attainable in vivo.

An unexpected observation in this study was that clavulanic acid alone inhibited inclusion formation. Clavulanic acid is not thought to have significant antibacterial activity against most organisms, although it does have activity against Legionella pneumophila, Neisseria gonorrhoeae, and Bacteroides fragilis (8). The effect of clavulanic acid was not likely due to direct toxicity on the McCoy cells, because monolayers remained intact and excluded trypan blue. Tests to detect more subtle abnormalities of the McCoy cells were not done. Despite the apparent activity of clavulanic acid, combining it with ticarcillin or amoxicillin did not enhance the activity of ticarcillin or amoxicillin against C. trachomatis.

Thus, these results support previous studies showing that penicillins have a definite but incomplete inhibitory effect on C. trachomatis in vitro. This study suggests that the addition of clavulanic acid to either ticarcillin or amoxicillin does not enhance activity against C. trachomatis in vivo. However, because ticarcillin and amoxicillin have a marked ability to decrease C. trachomatis replication, as indicated by the ≥99% decrease in the number of inclusions at readily attainable levels in serum, in most cases multiple-dose treatment suppresses and possibly even eradicates some C. trachomatis infections. However, the unpredictable and incomplete response should preclude use of these antimicrobial agents if the desire is to eradicate C. trachomatis. Rather, standard antimicrobial agents such as tetracyclines should be used for the treatment of C. trachomatis infections.

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LITERATURE CITED


TABLE 1. In vitro activity of antimicrobial agents against 21 C. trachomatis strains

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (μg/ml)</th>
<th>Median (range) concn (μg/ml) for 100% Decrease in inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticarcillin</td>
<td>&gt;960</td>
<td>1.875 (0.469–1.875)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>&gt;64 (64–&gt;64)</td>
<td>1 (0.25–1)</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>&gt;32 (32–&gt;32)</td>
<td>1 (0.25–16)</td>
</tr>
<tr>
<td>Ticarcillin-clavulanic acid</td>
<td>&gt;480/32 (480/32–&gt;480/32)</td>
<td>1.875/0.125 (0.469/0.031–1.875/0.125)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>&gt;32/8 (32/8–&gt;32/8)</td>
<td>0.5/0.125 (0.125/0.031–2/0.5)</td>
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