Antimicrobial Activity of Several Antibiotics and a Sulfonamide Against Chlamydia trachomatis Organisms in Cell Culture

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Minimum inhibitory concentrations of several antibiotics and a sulfonamide for growth of the 15 known immunotypes of Chlamydia trachomatis were determined in HeLa 229 cell cultures. The concentrations for complete inhibition of infectious-organism production were (per milliliter): tetracycline, 0.02 to 0.5 μg; rosinamic, 0.05 to 0.25 μg; erythromycin, 0.1 to 0.5 μg; chloramphenicol, 10 μg; penicillin, 0.02 to 50 U; ampicillin, 0.1 to 50 μg; and sulfisoxazole, 2 to 200 μg. The same concentrations of tetracycline, rosinamic, erythromycin, and chloramphenicol were sufficient to inhibit C. trachomatis inclusion formation. An increased concentration of sulfisoxazole was often needed to inhibit inclusion formation. Penicillin at 100 U/ml and ampicillin at 100 μg/ml failed to completely inhibit inclusion formation.

Recent development of improved cell culture methods for Chlamydia trachomatis (5, 9) has greatly simplified the in vitro testing of antimicrobial drugs against these organisms. In this report, the activities of six antimicrobial drugs known to be inhibitory against Chlamydia, both in natural infection and in vitro testing (7), were tested in HeLa 229 cell cultures (9) against C. trachomatis of all known immunological types. In addition, a new macroide antibiotic, rosinamic (12), was also tested.

MATERIALS AND METHODS

Chlamydia trachomatis strains. Seventeen C. trachomatis strains were tested. These included at least one of each of the 15 known C. trachomatis immunotypes. They were ocular trachoma strains: A/G-17/OT, B/TW-5/OT, Ba/AP-2/OT, C/TW-3/OT, C/ND-3/OT, D/G-1/OT; genital trachoma strains: E/ UW-5/Cx, F/UW-55/Ur, F/IOL-82/Ur, G/UW-57/Cx, H/UW-4/Cx, I/ICCal-6/ON, J/UW-36/Cx, K/UW-31/ Cx; and Lymphogranuloma venereum (LGV) strains: L1/440/Bu, L2/JH/Bu, and L3/404/Bu. The origins of these strains have been described (1, 8, 14-16). They are laboratory-established strains that have been serially passaged in eggs and/or HeLa cells.

Stock cultures were suspended in sucrose-potassium phosphate-glutamate buffer (9), divided in small portions, and frozen at −65°C. The titers of these inocula were 2 × 10⁶ to 10 × 10⁶ inclusion-forming units per ml by titration in HeLa 229 cells. One-tenth milliliter of inoculum usually gave 3 to 25 inclusions per micrometer field (7 by 7 mm) at x400 magnification in a light microscope. The same pools were used throughout the tests.

Antimicrobial agents. The following antimicrobial agents were tested, and their sources are listed below: (i) tetracycline, Tetracyn, Pfizer Inc., New York N.Y.; (ii) rosinamic, Schering Laboratories, Bloomfield, N.J., a new macroide antibiotic kindly supplied by Schering; (iii) erythromycin, Erythromycin lactobionate, Abbott Laboratories, North Chicago, Ill.; (iv) chloramphenicol, Chloromycetin sodium succinate, Parke, Davis & Co., Detroit, Mich.; (v) penicillin, potassium penicillin G, Eli Lilly & Co., Indianapolis, Ind.; (vi) ampicillin, Ampicillin, Schering, Schering-Plough Corp., New York, N.Y.; (vii) sulfonamide, Gantrisin (sulfisoxazole diolamine), Hoffman-La Roche, Inc., Nutley, N.J. Commercial preparations were used, except for rosinamic which was used in pure form. These agents, except rosinamic, have been known to inhibit chlamydiae (3, 6, 7).

The agents were placed into solution following label directions. They were further diluted with Hanks balanced salt solution to make a stock solution, divided into small portions, and frozen at −20°C. Gantrisin, which is in solution, was used unfrozen, and a new ampoule was used each time. For testing, stock solutions were further diluted and then added to the cell culture medium at desired concentrations. The concentrations of antimicrobial agents are expressed as micrograms per milliliter, with the exception of penicillin G expressed as units per milliliter.

Drug susceptibility test in cell culture. Our method for inoculating HeLa 229 cells with C. trachomatis strains has been described (9). A confluent 1-day-old HeLa 229 cell growth on a 12-mm circular cover slip in a 1-dram (ca. 3-ml) vial was used for inoculation. Before inoculation, the cell layer was treated with 30 μg of diethylaminoethyl-dextran per
ml (omitted with LGV strains). After removing diethylaminoethyl-dextran, 0.1 ml of inoculum was added, and the inoculated tubes were centrifuged at 900 × g for 60 min at 20°C. The inoculum was then removed; culture medium containing various amounts of the test drug was added, and the tube was incubated at 35°C for 3 days. No other antibiotics except the test drugs were incorporated in the culture medium. Four tubes were inoculated per drug dilution. The cover slips from two tubes were stained by Giemsa for detecting inclusions by whole-field scanning. The cells in the remaining two tubes were harvested, 0.5 ml/tube, and frozen at −65°C. The presence of organisms was determined by an inoculation into duplicate HeLa tubes, which were examined for inclusions. The results were expressed as the minimum inhibitory concentration (MIC) for viability (complete inhibition of inclusion formation) and the MIC for infectivity (complete inhibition of production of infectious organisms).

One or two trachoma strains were used for preliminary determinations of the MIC for each test drug. This concentration was then used to test the remaining strains. If the concentration was completely inhibitory, a fivefold less concentration was tested to see whether it was still inhibitory. If complete inhibition was not obtained with the predetermined concentration, a fivefold greater concentration was tested to determine the end point. Because of great strain variation and unsusceptibility to penicillin and ampicillin, only two additional concentrations at 50 and 100 U or µg/ml were tested if no inhibition was seen at 12.5 U or µg/ml. With sulfoxazole, which showed toxicity to the host HeLa cells at 1,000 µg/ml, the highest nontoxic concentration tested was 500 µg/ml.

RESULTS

Results of the drug susceptibility tests are summarized in Table 1.

All the antimicrobial agents tested were active against the C. trachomatis strains tested. Tetracycline, rosmarinic, erythromycin, and chloramphenicol showed narrow ranges of susceptibility. The MIC values for viability and infectivity for these antibiotics were the same or different within one fivefold dilution.

Both penicillin and ampicillin showed some wide variations in MIC values for the different strains. Strains C/TW-3, L1, and L2 were relatively unsusceptible to these antibiotics. H/UW-4 was not susceptible to ampicillin alone. When another trachoma type C strain (ND-3 from India) was tested, it was found to be highly susceptible to penicillin.

Neither penicillin nor ampicillin completely inhibited inclusion formation in the maximum concentration used. However, morphological changes in the inclusions could be seen with as little as 0.02 U of penicillin and 0.02 µg of ampicillin per ml. With both antibiotics, some amorphous eosinophilic material appeared in the inclusion with the higher antibiotic concentrations. Elementary body particles were not seen in the inclusion vacuoles.

Sulfoxazole also had a range of inhibitory concentrations. The genital trachoma strains (types E, F, G, H, I, J, and K) were more susceptible to sulfoxazole than the ocular trachoma (types A, B, Ba, and C) and LGV strains. The MIC values for viability were generally higher than for infectivity. In the vicinity of the infectivity MIC, the inclusions showed a decrease in number and size, and they contained fewer particles.

The antimicrobial agents at the effective concentrations did not show toxicity to the host HeLa cells. Penicillin and ampicillin at 100 U/ml and 100 µg/ml, respectively, were not toxic. Sulfoxazole was toxic at 1,000 µg/ml.

DISCUSSION

This is the first report in which all known immunotypes of C. trachomatis were tested for drug susceptibility. The results found in this study on the antichlamydial activity of tetracycline, erythromycin, chloramphenicol, penicillin, and sulfonamide were, in general, similar to those reported in earlier studies with animal models, embryonated eggs, or cell cultures (3, 6, 7, 11). The studies have found tetracycline and erythromycin to be the most effective, which is consistent with results of treatment of human infections.

C. trachomatis strain differences in susceptibility to tetracycline, penicillin, and sulfonamides have been reported from this laboratory based on tests with embryonated chicken egg yolk sac (11). The results in ovo and those reported here suggest that the differences observed are strain differences and not immunotype difference, the best example being the different susceptibility of the two type C strains tested. Such results make it necessary to test a number of different C. trachomatis strains before deciding on the activity of a drug against these organisms. Although the experiments were not set up to study definitively the difference in susceptibility of groups of trachoma strains, the results obtained suggested that, on the average, genital strains were more resistant to penicillin and more susceptible to sulfoxazole than ocular strains. The previous studies by Shiao et al. (11) in ovo found similar results with penicillin. The finding that genital strains were more resistant to penicillin was attributed to possible selection of penicillin-resistant strains due to increased usage of this antibiotic in persons with venereal diseases. Penicillin-resistant
variants of \textit{Chlamydia} have been produced in the laboratory by prolonged passages of sensitive strains in eggs containing subinhibitory concentrations of penicillin (4, 7, 10).

Penicillin’s effect on chlamydial inclusions is well known. While the antibiotic clearly causes abnormal growth patterns, it is difficult to eradicate the organism with a reasonable dosage. In experiments with mice, doses of penicillin that prevented death of infected mice were not effective in eradication of the organisms from the host (2, 7). In embryonated eggs much larger amounts of penicillin were required to completely inhibit chlamydial replication than to protect eggs from infectious death (7). These findings have been associated with the clinical observation that penicillin is relatively ineffective in treatment of \textit{C. trachomatis} infections.

Rosamicin has broad-spectrum in vitro activity against gram-positive and gram-negative bacteria as well as mycoplasma and anaerobe species (12, 13). The antimicrobial activity is comparable to erythromycin against gram-negative bacteria. This study showed that rosamicin is also active against \textit{C. trachomatis} strains. It is as effective as tetracycline and is 2 to 10 times more effective by weight than erythromycin.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


**TABLE 1. MIC values of antibiotics and sulfonamide for \textit{Chlamydia trachomatis} in cell cultures**

<table>
<thead>
<tr>
<th>C. \textit{trachomatis} strain</th>
<th>Tetra-cycline</th>
<th>Rosamicin</th>
<th>Erythromycin</th>
<th>Chloramphenicol</th>
<th>Penicillin</th>
<th>Ampicillin</th>
<th>Sulfisoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/G-17/OT</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
<td>10</td>
<td>0.02(&gt;100)</td>
<td>0.1(&gt;100)</td>
<td>200</td>
</tr>
<tr>
<td>B/TW-5/OT</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
<td>10</td>
<td>0.02(&gt;100)</td>
<td>0.1(&gt;100)</td>
<td>200</td>
</tr>
<tr>
<td>Ba/AP-2/OT</td>
<td>0.1</td>
<td>0.05</td>
<td>0.5</td>
<td>10</td>
<td>0.02(&gt;100)</td>
<td>0.5(&gt;100)</td>
<td>200</td>
</tr>
<tr>
<td>C/TW-3/OT</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
<td>10</td>
<td>12.5(&gt;100)</td>
<td>50(&gt;100)</td>
<td>200</td>
</tr>
<tr>
<td>C/ND-3/OT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>0.02(&gt;100)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>D/G-1/OT</td>
<td>0.02</td>
<td>0.05</td>
<td>0.1</td>
<td>10</td>
<td>0.1(&gt;100)</td>
<td>0.1(&gt;100)</td>
<td>200</td>
</tr>
<tr>
<td>E/UW-5/Cx</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
<td>10</td>
<td>0.1(&gt;100)</td>
<td>0.1(&gt;100)</td>
<td>40(500)</td>
</tr>
<tr>
<td>F/UW-55/Ur</td>
<td>0.02</td>
<td>0.05</td>
<td>0.1</td>
<td>10</td>
<td>0.1(&gt;100)</td>
<td>0.5(&gt;100)</td>
<td>8</td>
</tr>
<tr>
<td>G/IOL-82/Ur</td>
<td>0.02</td>
<td>0.05</td>
<td>0.1</td>
<td>10</td>
<td>0.1(&gt;100)</td>
<td>0.5(&gt;100)</td>
<td>40</td>
</tr>
<tr>
<td>G/UW-57/Cx</td>
<td>0.1</td>
<td>0.05</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>40(500)</td>
</tr>
<tr>
<td>H/UW-4/Cx</td>
<td>0.1</td>
<td>0.05</td>
<td>0.5</td>
<td>10</td>
<td>0.1(&gt;100)</td>
<td>50(&gt;100)</td>
<td>40(500)</td>
</tr>
<tr>
<td>I/CCal-6/ON</td>
<td>0.1</td>
<td>0.05</td>
<td>0.5</td>
<td>10</td>
<td>0.1(&gt;100)</td>
<td>0.5(&gt;100)</td>
<td>2</td>
</tr>
<tr>
<td>J/UW-36/Cx</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
<td>10</td>
<td>0.5(&gt;100)</td>
<td>2.5(&gt;100)</td>
<td>40</td>
</tr>
<tr>
<td>K/UW-31/Cx</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
<td>10</td>
<td>0.5(&gt;100)</td>
<td>2.5(&gt;100)</td>
<td>40</td>
</tr>
<tr>
<td>L/440/Bu</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>10</td>
<td>50(&gt;100)</td>
<td>50(&gt;100)</td>
<td>200</td>
</tr>
<tr>
<td>L/404/Bu</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
<td>10</td>
<td>12.5(&gt;100)</td>
<td>50(&gt;100)</td>
<td>200</td>
</tr>
<tr>
<td>L/404/Bu</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
<td>10</td>
<td>2.5(&gt;100)</td>
<td>0.5(&gt;100)</td>
<td>40</td>
</tr>
</tbody>
</table>

* MIC for infectivity; MIC for viability is given in parentheses only if it differed more than fivefold from MIC for infectivity.

* Highest concentration tested.

* NT, Not tested.


