In Vitro Drug Susceptibility of *Chlamydia* sp. Strain TWAR

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The in vitro susceptibilities of eight *Chlamydia* sp. strain TWAR isolates were tested against tetracycline, erythromycin, penicillin, ampicillin, sulfisoxazole, and a new drug, trospectomycin. The ranges of inhibitory concentrations of these antimicrobial agents, except for sulfonamide, were similar to those for *Chlamydia trachomatis*. Sulfisoxazole was not inhibitory at the highest nontoxic concentration tested.

We recently described a new chlamydial strain, TWAR, associated with acute respiratory diseases in humans (3, 4, 7; M. Kleemola, P. Saikkku, R. Visakorpi, S.-P. Wang, and J. T. Grayston, J. Infect. Dis., in press). Epidemiological studies have shown that TWAR causes 6 to 12% of community-acquired cases of pneumonia in North America (3, 7). Serological, DNA, and ultrastructural studies have shown that the TWAR agent is a unique *Chlamydia* strain (1–4). Since it is a newly recognized *Chlamydia* strain, different from *Chlamydia psittaci* and *Chlamydia trachomatis*, we tested its in vitro susceptibilities to several commonly used antimicrobial chemotherapeutic agents and a spectominycin analog, trospectomycin, in cell culture.

Eight TWAR strains, including TW-183, AR-39, AR-231, AR-277, AR-388, AR-427, AR-458, and LR-65, were tested (3, 4). TW-183 is an ocular isolate obtained in Taiwan in 1965. The remaining strains were isolated in Seattle from 1983 to 1986 from the throats of adults with acute respiratory diseases. These strains had been through 15 to 23 passages in HeLa 229 cell cultures.

The antimicrobial agents tested were tetracycline (Achromycin, intravenous; Lederle Laboratories, Pearl River, N. Y.), erythromycin (Erythromycin Lactobionate; Abbott Laboratories, North Chicago, Ill.), and sulfisoxazole (Sigma Chemical Co., St. Louis, Mo.), and ampicillin (Polycillin-N; Bristol Laboratories, Syracuse, N. Y.), penicillin (Penicillin G potassium; Pfizer Inc., New York, N. Y.), and trospectomycin (6'-n-propylspectinomycin sulfate, crystalline; The Upjohn Co., Kalamazoo, Mich.).

Achromycin, Erythromycin, Penicillin G, and Polycillin-N were reconstituted in solution by following label directions. Trospectomycin was dissolved in distilled water. Sulfisoxazole was first dissolved in 0.1 M NaOH and then diluted with distilled water. Stock solutions were prepared by further dilution with Hanks balanced salt solution, divided into small portions, and frozen at −20°C. Immediately before being tested the vials were thawed and the antibiotics were added to the culture medium (Eagle minimal essential medium containing 10% fetal calf serum) to the desired concentrations. A fresh vial was used for each test.

Susceptibility to drug was tested in cell culture by our published method (5) with minor modifications (4). HeLa 229 cells grown on a cover slip in a vial shell were pretreated with 30 µg of DEAE-dextran per ml. Inoculum (0.1 ml) was added. Inoculum concentrations were adjusted to yield two to five inclusions per field (∼400 magnification) in controls, i.e., 0.1 ml of a 10−2 to 10−4 dilution. Vials were centrifuged at 2,200 rpm (900 × g) for 60 min at room temperature. The inoculum was removed. Culture medium (1 ml) containing 0.5 µg each of cycloheximide and test drug was added. The cultures were incubated at 35°C for 3 days. Two cover slips were fixed with methanol and stained with fluorescein-conjugated genus-specific monoclonal antibody CF-2 by our published method (4, 9). The stained cells were examined under a fluorescence microscope for inclusions. Cells for passage 2 were taken from the remaining two culture vials and cultured without antibiotics. For passages, the culture medium was reused. Cells from two vials were harvested in 0.3 ml of *Chlamydia* transport sucrose-phosphate-glutamic acid medium by scraping with a Pasteur pipette. Two new vials were inoculated as described above. After incubation, cells were stained with the same fluorescein conjugate and examined for inclusions. Antibiotics were tested in serial twofold dilutions. The MICs for the complete inhibition of inclusion formation in the original inoculation (MIC for viability) and for the complete inhibition of inclusion formation in passage 2 (MIC for infectivity) were determined.

The MICs of test drugs for eight TWAR isolates are given in Table 1. All eight TWAR isolates tested showed homogenous susceptibility to tetracyclines and macrolides. MICs for both viability and infectivity were equal or within 1 twofold dilution of each other. Penicillin and ampicillin were also effective in inhibiting infectivity. However, a concentration as high as 100 U or 100 µg, respectively, per ml failed to suppress the viability, i.e., failed to suppress inclusion formation in the original inoculation. These inclusions were atypical, with a honeycomb appearance. A concentration as low as 0.05 U of penicillin or 0.1 µg of ampicillin per ml was shown to produce changes in inclusion morphology. As drug concentrations were increased, the inclusions became smaller and empty, often with a signet ring appearance.

Sulfisoxazole failed to inhibit *Chlamydia* sp. strain TWAR at 400 µg/ml, except for two strains (AR-277 and LR-65), which were inhibited at this concentration. Sulfisoxazole was toxic to HeLa cells at 600 µg/ml.

Trospectomycin also showed an inhibitory effect on TWAR organisms. Both MICs were equal or differed within 2 twofold dilutions.

Drug toxicity in HeLa cells was not observed within the described dosage ranges, except with sulfisoxazole, which showed toxicity at 600 µg/ml.

TWAR strains showed susceptibility like that of *C. trachomatis* to tetracyclines, macrolides, and penicillins. Using a method similar to that used with TWAR strains, we had previously found that the susceptibility of trachoma and LGV biotypes of *C. trachomatis* to tetracycline was in the...
TABLE 1. MICs for Chlamydia sp. strain TWAR

<table>
<thead>
<tr>
<th>Drug</th>
<th>Viability (µg/ml or U/ml)</th>
<th>Infectivity (µg/ml or U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>0.05–0.1</td>
<td>0.05–0.1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.01–0.05</td>
<td>0.01–0.05</td>
</tr>
<tr>
<td>Penicillin</td>
<td>&gt;100</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;100</td>
<td>0.8–1.6</td>
</tr>
<tr>
<td>Trospectomycin</td>
<td>10–20</td>
<td>5–40</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>≥400</td>
<td>≥400</td>
</tr>
</tbody>
</table>

* The following eight strains of Chlamydia sp. strain TWAR were tested: TW-183, AR-39, AR-231, AR-277, AR-388, AR-427, AR-458, and LR-65.
* For viability, MICs that completely inhibit inclusion formation in the original passage; for infectivity, MICs that completely inhibit production of infectious organisms as determined by passage 2.
* MICs for Chlamydia sp. were tested: AR-277, LR-65 were inhibited at 400 µg/ml.

According to many laboratory and clinical studies with C. trachomatis and our limited information from clinical studies with Chlamydia sp. strain TWAR (3; Kleemola et al., in press), erythromycin and tetracyclines appear to be the drugs of choice for treatment of TWAR infection. However, further clinical studies should be done to determine the drug efficacy and the dosage and duration of drug administration.

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