Antimicrobial Susceptibility of Intracellular *Legionella pneumophila*

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Minimum inhibitory concentrations, minimum bactericidal concentrations, and killing curve assays were measured after *Legionella pneumophila* was grown in human lung fibroblasts. Results of susceptibility testing of organisms grown intracellularly are compared with results for organisms grown by standard tube dilution methods.

In vitro tests have shown that *Legionella pneumophila* serotype 1 is susceptible to a variety of agents (3, 8-10). In clinical studies, patients treated with erythromycin have low mortality rates and fast recovery time, making this the drug of choice for treating *L. pneumophila* infections (4). For many antibiotics, in vitro antimicrobial susceptibility of *L. pneumophila* correlates poorly with in vivo results (3, 5, 7). In particular, gentamicin appears highly effective in vitro, but in guinea pigs and humans, this drug appears to have little activity (5, 7-9). As *L. pneumophila* is an intracellular pathogen (1, 2, 6), we attempted to determine whether testing antimicrobial susceptibility of *L. pneumophila* in an intracellular environment rather than on agar plates would produce results which correlate better with in vivo experience.

*L. pneumophila* has been shown to replicate within human embryonic lung fibroblast cells (2, 12). We chose to study the effects of antibiotics on intracellular multiplication of *L. pneumophila* within these cells. The fibroblasts were grown in Eagle minimal essential medium supplemented with 0.025% bicarbonate–1% glutamine–7.5% fetal bovine serum. The cells were infected with 10⁶ colony-forming units of *L. pneumophila* serotype 1 (ATCC 33152). At 2.5 h postinfection, various concentrations of erythromycin (CIBA Pharmaceutical Company, Summit, N.J.), gentamicin (Schering Laboratories, Bloomfield, N.J.), or cefamandole (Eli Lilly & Co., Indianapolis, Ind.) were added to the tissue culture medium. At 72 h postinfection, the fibroblast monolayer was washed with phosphate-buffered saline, the supernatant fluids were discarded, and the cells were scraped into 1 ml of phosphate-buffered saline. The titer of *L. pneumophila* within the cells was determined by counting colony growth on charcoal-yeast extract agar. Tissue culture monolayers were inoculated at 37°C in 5% CO₂, and titrations on charcoal-yeast extract agar were performed at 37°C in 2.5% CO₂. We previously reported that *L. pneumophila* does not replicate in Eagle minimal essential medium alone unless a cell monolayer is present (2).

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the various antibiotics were determined in Feeley-Gorman broth by a standard tube dilution method (11). In the fibroblast tissue culture system, the lowest antibiotic concentration allowing no turbid growth in the tissue culture medium determined the MIC, and the lowest antibiotic concentration which resulted in at least a 3-log decrease in growth determined the MBC. Table 1 summarizes the antimicrobial susceptibility results.

Erythromycin and gentamicin were effective in inhibiting and killing intracellular *L. pneumophila*, whereas cefamandole was ineffective. Similar results were obtained by testing antimicrobial susceptibility in tissue culture and broth systems. Significant differences were not demonstrated between the susceptibilities of *L. pneumophila* to erythromycin and gentamicin in the tissue culture system. To discover if one antibiotic was a more rapid and efficient bactericidal agent, we determined killing curves for these two antibiotics.

Killing curve methodology paralleled MIC/MBC procedures described earlier. The MBC of each antibiotic determined the antibiotic concentration used in the tissue culture medium (erythromycin, 1 μg/ml; gentamicin, 0.5 μg/ml).
Harvests of cells for titration of viable organisms were performed immediately after the adsorption period (2.5 h) and at 4, 24, 48, and 72 h later. As shown in Fig. 1, gentamicin was a more rapid bactericidal agent than erythromycin. After 4 h, no L. pneumophila was recovered from the gentamicin-treated fibroblasts. L. pneumophila titers in the erythromycin-treated fibroblasts gradually increased for the first 48 h and then declined to 0 by 72 h. Growth in controls which contained no antibiotics markedly increased by 48 and 72 h postinfection.

These results indicate that antimicrobial susceptibility testing can be performed in a tissue culture system. However, this method does not correlate any better with in vivo observations for gentamicin efficiency than previously reported broth or agar systems. Horwitz and Silverstein (6) recently reported that L. pneumophila replicates intracellularly in human monocytes. Perhaps testing antimicrobial susceptibility in monocytes will better approximate in vivo observations, as the L. pneumophila appears to reside in monocytes during infection (1). Alternatively, as no prospective studies have been performed to examine gentamicin efficacy in vivo, the use of this drug in only the most ill patients may be skewing the interpretation of its efficacy.

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