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In Vitro Activity of Azithromycin against Clinical Isolates of *Legionella* Species

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The activities of azithromycin, erythromycin, and ciprofloxacin against 21 *Legionella* isolates were measured by an agar dilution method and in macrophages. The MICs for 90% of strains tested were 2.0, 1.0, and 0.5 μg/ml for azithromycin, erythromycin, and ciprofloxacin, respectively. Azithromycin and ciprofloxacin were both bactericidal in the macrophage system, but erythromycin was bacteriostatic.

Azithromycin is a new macrolide antibiotic that is active against intracellular pathogens and attains very high and persistent intracellular concentrations (1, 2, 6–9). We tested *Legionella* spp. with azithromycin by using a variety of susceptibility testing methods designed to determine whether the pharmacokinetic advantages of the drug are accompanied by activity against *Legionella* spp.

All legionellae studied were clinical isolates. These strains were identical to those used in a prior study and included one strain of *L. bozemanii*, two strains of *L. dumoffii*, two strains of *L. longbeachae*, two strains of *L. micdadei*, and 14 strains of *L. pneumophila* (4). *Staphylococcus aureus* ATCC 29213 was used as a control organism for susceptibility testing. Legionellae were grown on locally made buffered charcoal-yeast extract medium supplemented with 0.1% α-ketoglutarate (BCYEα) (3). Incubation of all media was at 35°C in humidified air. Standard powers of azithromycin, erythromycin, and ciprofloxacin were obtained from their manufacturers.

Agar dilution susceptibility testing was performed as described previously (4). Briefly, antimicrobial agent-containing BCYEα agar plates were inoculated with $\approx 10^5$ CFU of bacteria. The control *S. aureus* strain was inoculated to antimicrobial agent-containing Mueller-Hinton agar plates as well as to BCYEα plates to determine whether BCYEα medium inhibited antimicrobial agent activity. The plates were incubated for either 24 h (*S. aureus*) or 48 h (legionellae), at which time MICs were determined. Macrobroth dilution susceptibility testing was performed with *L. pneumophila* F889 and F2111 as previously described (4). All testing was done in duplicate; in the case of disagreement, the geometric mean value was used as the MIC. Erythromycin and ciprofloxacin were included as controls; data for the activity of these two drugs against the *Legionella* strains we tested have been presented previously (4).

Guinea pig pulmonary alveolar macrophages were harvested and purified as described previously (4). The final concentration of macrophages was approximately 10^5 cells per well. Antimicrobial agent susceptibility testing of intracellular *L. pneumophila* was performed as described previously (4). Briefly, $\approx 10^4$ CFU of washed BCYEα plate-grown *L. pneumophila* was added to the purified alveolar macrophages. The bacteria and macrophages were incubated for 1 day after 1 h of shaking incubation. Antimicrobial agents were added to wells after the wells had been washed three times to remove nonadherent bacteria. Sonic extracts from two replicate, non-antimicrobial-agent-containing wells were quantitatively cultured for use as the day 1 bacterial count. Non-antibiotic-containing wells were used as growth controls. After 2 days of incubation, the supernatants were sampled and quantitatively cultured; all wells were then washed to remove antimicrobial agents. Bacterial counts in the supernatant of each well were determined for another 4 or 5 days. All experiments and quantitative plating were carried out in duplicate.

The MIC for 50% of *Legionella* strains was 0.5 μg/ml for all three antimicrobial agents. The MICs for 90% of strains were 2.0, 1.0, and 0.5 μg/ml for azithromycin, erythromycin, and ciprofloxacin, respectively. All three antimicrobial agents were inhibited by BCYEα agar, as determined by their MICs for the control *S. aureus* strain with BCYEα and Mueller-Hinton agar media. The azithromycin MIC for *S. aureus* was 3 $\log_2$ greater with BCYEα agar than with Mueller-Hinton agar. The ciprofloxacin MIC for *S. aureus* was 1.5 $\log_2$ dilutions greater with BCYEα agar, and the erythromycin MIC was 1 $\log_2$ dilution greater. BYEα broth was much less inhibitory than was BCYEα agar for azithromycin and ciprofloxacin but not for erythromycin. The azithromycin and erythromycin MICs for *S. aureus* were both 1 $\log_2$ dilution greater with BCYEα agar than with Mueller-Hinton broth. The ciprofloxacin MIC was the same with both BCYEα and Mueller-Hinton media. Agar and broth dilution MICs for strains F2111 and F889 are shown in Table 1.

The two *L. pneumophila* serogroup 1 strains behaved similarly in the guinea pig alveolar macrophage system, so only the data for strain F2111 are shown in Fig. 1 and 2. Both strains were inhibited by all three antibiotics after 48 h of incubation. High (5 μg/ml) but not low (1 μg/ml) concentrations of azithromycin prevented any regrowth for 5 days after the antimicrobial agents were washed out. The same was true with ciprofloxacin (1 μg/ml) and strain F889 but not with F2111.

In the guinea pig alveolar macrophage model, azithromycin and ciprofloxacin were apparently bactericidal for *L. pneumophila*, unlike erythromycin, which was purely static. The prolonged activity of azithromycin in this system is likely due to its high and prolonged intracellular concentration within the macrophage (7). These studies do not estab-

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TABLE 1. Agar and broth macrodilution susceptibilities of strains F889 and F2111

L. pneumophila strain and drug	MIC (μg/ml)	
	BCYEα agar	BYEα broth
F889		
Azithromycin	0.25	0.06
Erythromycin	0.5	0.125
Ciprofloxacin	0.5	0.06
F2111		
Azithromycin	1.5	0.125
Erythromycin	1.0	0.125
Ciprofloxacin	≤0.03	0.06

lish whether the prolonged postantibiotic effect observed for azithromycin (5 μg/ml) is the sole result of intracellular drug persistence or of some true bactericidal activity as well. Serum azithromycin concentrations in humans after a single 500-mg dose peak at 0.5 μg/ml and fall to 0.1 μg/ml after 10 h (6), levels which are higher than the extracellular broth MICs measured in these studies. It is very unlikely that concentrations of 5 μg/ml in serum could be obtained in humans with usual drug dosages, but the high drug levels found in lung (5 μg/ml) and in cells (10 to 70 μg/ml) make this drug likely to be effective for the treatment of Legionnaires disease (6, 7, 9). Azithromycin will probably be more effective than erythromycin for the treatment of Legionnaires disease, as has been demonstrated in an animal model, warranting human clinical trials with this drug (5).

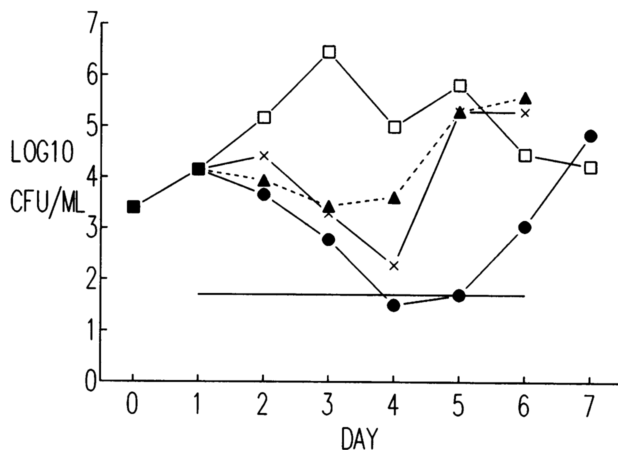

FIG. 1. Growth of *L. pneumophila* serogroup 1 strain F2111 in guinea pig alveolar macrophages versus day of infection. The horizontal line represents the lower limit of bacterial detection. Symbols: □, growth control wells; ▲ and ×, wells containing 1.0 and 5.0 μg of erythromycin per ml, respectively; ●, wells containing 1.0 μg of azithromycin per ml.

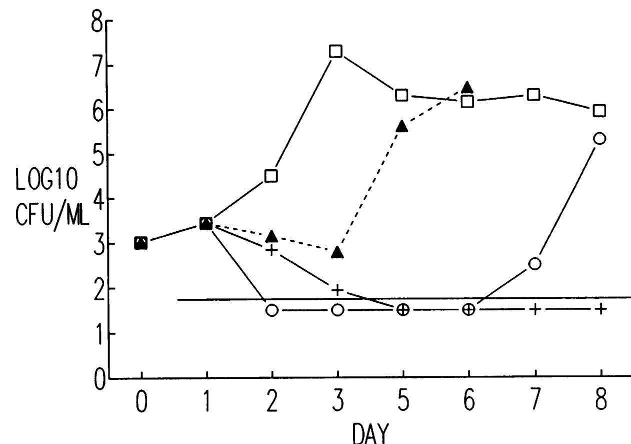

FIG. 2. Growth of *L. pneumophila* serogroup 1 strain F2111 in guinea pig alveolar macrophages versus day of infection. Symbols: □, growth control wells; ▲, wells containing 1.0 μg of erythromycin per ml; ○, wells containing 1.0 μg of ciprofloxacin per ml; +, wells containing 5.0 μg of azithromycin per ml.

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