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In Vitro Activity of Antimicrobial Agents on Legionnaires Disease Bacterium

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Six isolates of Legionnaires disease bacteria were tested for their susceptibility to 22 antimicrobial agents. The most active agent was rifampin (minimal inhibitory concentration, ≤0.01 μg/ml). On the basis of minimal inhibitory concentration breakpoints that have been used to categorize susceptibility for most of these drugs, the organisms were susceptible to rifampin, cefoxitin, erythromycin, the aminoglycosides, minocycline and doxycycline, chloramphenicol, ampicillin, penicillin G, carbenicillin, colistin, and sulfamethoxazole-trimethoprim (19:1 ratio); sensitive to intermediate in susceptibility to tetracycline, methicillin, cefamandole, cephalothin, and clindamycin; and resistant to vancomycin. More clinical data must be obtained before an optimal therapeutic regimen can be recommended.

In 1976 an outbreak of severe respiratory illness occurred in some attendees of an American Legion Convention in Philadelphia, Pa. (2). An estimated 180 persons were sick and 29 of them died (3). The etiological agent has been shown to be a bacterium (3), called the Legionnaires disease bacterium. There is now evidence that this organism was responsible for outbreaks of respiratory disease in the District of Columbia in 1965 (5) and in Pontiac, Mich., in 1968 (4). A similar organism has been isolated from frozen material saved from the Pontiac outbreak and from the pleural fluid of a patient in Flint, Mich., who later died (5).

This is a report on the in vitro activity of 22 antimicrobial agents against Legionnaires disease bacterium.

MATERIALS AND METHODS

Cultures. Six organisms were studied: four isolates were from the Philadelphia, Pa., outbreak of 1976; one isolate was recently obtained from pleural fluid of a patient who had died of pneumonia in Flint, Mich.; and one isolate was from the Pontiac, Mich., outbreak. The latter organism was grown from a lung nodule of a guinea pig that developed pneumonia during exposure in the Health Department building in Pontiac, Mich., in 1968. The lung nodule, frozen since 1968, was thawed and inoculated intraperitoneally into guinea pigs. Parts of spleens of guinea pigs were inoculated into yolk sacs of embryonated hen eggs. The bacterium was isolated from a yolk sac. These isolates were obtained from R. E. Weaver, Special Bacteriology Section, Center for Disease Control. The organisms were grown on Mueller-Hinton agar (BBL) supplemented with 1% IsoVitaleX (BBL) and 1% bovine hemoglobin (Difco) (MHIH agar).

Antimicrobial agents. The antimicrobial agents were supplied by the following organizations: penicillin G, methicillin, kanamycin, and amikacin, Bristol Laboratories, Syracuse, N.Y.; rifampin, CIBA Pharmaceutical Co., Summit, N.J.; carbenicillin, streptomycin, doxycycline, and vancomycin, Pfizer, Inc., New York, N.Y.; chloramphenicol, Parke, Davis, and Co., Detroit, Mich.; tetracycline and minocycline, Lederle Laboratories, Pearl River, N.Y.; ampicillin, Wyeth Laboratories, Philadelphia, Pa.; cefoxitin, Merck Institute, Rahway, N.J.; cephalothin, cefamandole, erythromycin, Eli Lilly and Co., Indianapolis, Ind.; clindamycin, Upjohn Co., Kalamazoo, Mich.; gentamicin, Schering Corp., Bloomfield, N.J.; colistin, Warner-Lambert Pharmaceutical Co., Morris Plains, N.J.; and sulfamethoxazole-trimethoprim, Burroughs-Wellcome, Research Triangle, N.C.

Susceptibility tests. Susceptibility tests were performed by agar dilution, with a Steers replicator (9). The medium was MHIH agar supplemented with appropriate concentrations of the antimicrobial agents listed above. For the inocula, 36-h growth was taken from MHIH agar, suspended in Mueller-Hinton broth, and diluted to contain approximately 10^9, 10^8, and 10^7 colony-forming units (CFU) per ml by comparing them with McFarland standards. The antibiotic-containing plates were dried and inoculated in duplicate with the replicator. Each inoculum "spot" contained approximately 10^4, 10^5, or 10^6 CFU, depending upon the initial inoculum used. The inocula were allowed to dry, and the plates were incubated in a candle extinction jar for 48 h at 35°C. The minimal inhibitory concentration (MIC) was the least concentration of antibiotic that prevented macroscopic growth of the organisms.

RESULTS

In general, the effect of the three different inocula on the susceptibility results was quite

minimal. Except for tests with clindamycin, the larger inoculum occasionally yielded an MIC one dilution higher than the mode, and, likewise, the lower inoculum occasionally yielded an MIC one dilution lower. However, results for clindamycin were more difficult to reproduce. MIC values obtained with an initial inoculum of 10^9 CFU per ml were two to four dilutions higher than when 10^7 CFU per ml was used, and an inoculum of 10^8 CFU per ml was one dilution higher. The MIC values shown in Table 1 were obtained with the initial inoculum of 10^8 CFU per ml.

MIC values for each isolate-drug combination and geometric mean MICs are shown in Table 1. The most active agent was rifampin, with an MIC of ≤ 0.01 μg/ml for each organism. On the basis of reported MIC break points for most of the drugs (10), the organisms could be judged susceptible to rifampin, cefoxitin, erythromycin, the aminoglycosides, minocycline, doxycycline, chloramphenicol, ampicillin, penicillin G, carbenicillin, colistin, and sulfamethoxazole-trimethoprim (19:1 ratio); sensitive to intermediate in susceptibility to tetracycline, methicillin, cefamandole, cephalothin, and clindamycin; and resistant to vancomycin. The isolates from the Philadelphia outbreak (no. 1, 2, 3, and 4) and the isolate from Flint, Mich. (no. 5) were quite similar in their susceptibility to the drugs, but the Pontiac strain (no. 6) was more susceptible to some drugs, particularly the penicillins.

DISCUSSION

Case records of 94 patients hospitalized with Legionnaires disease, including 26 who died, have been reviewed for the therapeutic regimens used to treat these patients (5). The case-fatality rate was higher with those patients who received cephalothin and lower for those treated with erythromycin or tetracycline.

Two studies on the in vitro activity of some antimicrobial agents on infection with the Legionnaires organism have been made. In one study, erythromycin was shown to be efficacious in curing guinea pigs infected with the organism; all infected guinea pigs that were not treated died (6).

In the second study (V. J. Lewis and W. L. Thacker, personal communication), chicken embryos were protected from the infection when either rifampin, gentamicin, streptomycin, erythromycin, sulfadiazine, chloramphenicol, or cephalothin was given before the infecting dose of organisms. Rifampin and erythromycin also protected against embryo death if given at 72 h after the infecting dose, and the

TABLE 1. *MICs of 22 antimicrobial agents on six isolates of the Legionnaires disease bacterium*

Antimicrobial agent	MIC (μg/ml) for organism						
	1[a]	2	3	4	5	6	GM[b]
Penicillin	2.0	2.0	1.0	1.0	2.0	0.5	1.30
Ampicillin	1.0	1.0	1.0	1.0	2.0	0.25	0.90
Carbenicillin	4.0	4.0	2.0	4.0	4.0	≤0.5	2.60
Methicillin	8.0	8.0	4.0	4.0	8.0	1.0	4.80
Cephalothin	16.0	8.0	4.0	32.0	32.0	32.0	16.0
Cefamandole	16.0	16.0	8.0	4.0	16.0	4.0	9.60
Cefoxitin	0.25	0.12	0.12	0.12	0.12	0.06	0.12
Amikacin	0.5	0.5	0.5	0.5	0.5	0.5	0.50
Gentamicin	0.5	0.12	0.25	0.12	0.12	0.25	0.20
Kanamycin	2.0	1.0	2.0	2.0	1.0	0.5	1.30
Streptomycin	2.0	1.0	2.0	1.0	1.0	1.0	1.30
Tobramycin	0.5	0.25	0.5	0.5	0.25	0.25	0.36
Erythromycin	0.25	0.12	0.12	0.12	0.5	0.12	0.18
Clindamycin	8.0	16.0	4.0	4.0	8.0	8.0	7.2
Chloramphenicol	0.5	0.5	0.5	0.5	0.5	0.5	0.50
Tetracycline	8.0	8.0	4.0	4.0	4.0	4.0	5.2
Minocycline	0.5	0.5	0.5	0.25	0.25	0.5	0.43
Doxycycline	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Rifampin	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤.01
Vancomycin	32.0	32.0	32.0	16.0	32.0	16.0	32.0
Colistin	4.0	4.0	4.0	2.0	4.0	4.0	3.6
Sulfamethoxazole-trimethoprim	4.8/0.25	4.8/0.25	4.8/0.25	4.8/0.25	4.8/0.25	4.8/0.25	4.8/0.25

[a] Isolates 1 through 4 were from the Philadelphia 1976 outbreak; isolate 5 was from the Flint, Mich., patient; and isolate 6 was from the Pontiac, Mich., 1968 outbreak.

[b] Geometric mean.

other drugs protected when given at 48 h if the dosage were increased twofold. Ampicillin and chlortetracycline did not protect in the dosages used.

Recently, a patient with Legionnaires disease was initially treated unsuccessfully with cephalothin, but was cured when he was treated with erythromycin (500 mg, intravenously, every 6 h) and gentamicin (80 mg, intravenously, every 8 h). Within 24 h his temperature fell from 103°F (ca. 39.4°C) to less than 100°F (ca. 37.8°C), and then he had an uneventful recovery (7).

All of the data cited above seem to indicate that erythromycin might be an effective agent for treating patients with Legionnaires disease. Our in vitro data support this hypothesis. Much more data must be collected, however, before the optimal antimicrobial regimen for therapy can be determined.

Our in vitro data, for the most part, agree with the results of the chicken embryo protection studies. In both studies, rifampin was the most active agent, and erythromycin, aminoglycosides, sulfonamides (in our case, sulfamethoxazole in combination with trimethoprim), and choloramphenicol were very active. Cephalothin in high dosages protected the embryos, and our cephalothin MIC values indicate that high dosages would be necessary. Although the other investigators found that ampicillin did not protect chicken embryos, we would have predicated that penicillins would have been more effective than cephalothin (and cefamandole) if used in higher doses. The chicken embryos were also not protected by chlortetracycline but were partially protected by oxytetracycline. The tetracycline MIC values from our study indicate that the organisms are probably moderately resistant to tetracycline. Based on the MICs, the most active β-lactam antibiotic was cefoxitin, a cefamycin, which has not been approved for general use. The penicillins had lower MIC values than cephalothin or cefamandole, but the MICs were still relatively high, and the penicillins would probably be required in high dosages if used in therapy.

Although the MIC values for tetracycline were relatively high (4 to 8 μg/ml), minocycline and doxycycline were active (MIC, 0.25 to 1 μg/ml). Differences in the activities of these drugs for some groups of organisms have been previously reported, e.g., increased activity of minocycline on strains of *Acinetobacter* and *Serratia* (8).

The only notable difference between the susceptibility data for these six isolates is that isolate 6 (the Pontiac strain) is more susceptible to the penicillins. Whether this was a factor in the absence of death among patients with "Pontiac fever" can only be a subject for conjecture. Even though these MIC values and other data suggest that erythromycin and possibly the aminoglycosides are efficacious agents in the treatment of patients with Legionnaires disease, the data are inadequate to recommend an optimal therapeutic regimen. Furthermore, therapy with appropriate drugs may fail even though the MICs are low, e.g., penicillin may not cure some patients with pneumonia caused by certain types of pneumococci, even though the organisms are quite susceptible (1). Solely on the basis of these in vitro data, however, the best drugs for prospective clinical trials may be rifampin, cefoxitin, erythromycin, the aminoglycosides, chloramphenicol, minocycline, doxycycline, and sulfamethoxazole-trimethoprim. The penicillins and cephalosporins would probably require high dosages.

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