Comparison of In Vitro Antimicrobial Susceptibilities of *Mycobacterium avium-M. intracellulare* Strains from Patients with Acquired Immunodeficiency Syndrome (AIDS), Patients without AIDS, and Animal Sources

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Difloxacin, A-56620, cefazolin, cefotaxime, ceftizoxime, cephapirin, SK&F 88070, and spectinomycin were used to compare the in vitro susceptibilities of *Mycobacterium avium-M. intracellulare* isolates from patients with acquired immunodeficiency syndrome (AIDS), patients without AIDS, and diseased animals. Against the isolates from humans without AIDS, the quinolone compounds difloxacin and A-56620 were found to be the most effective, each inhibiting 50% of strains at a concentration of 2 μg/ml. The remaining antimicrobial agents had MICs for 50% of strains tested of at least 32 μg/ml. Statistically significant differences were observed in the antibiogram patterns among the *M. avium-M. intracellulare* strains from each of the three sources.

Since most *Mycobacterium avium-M. intracellulare* (MAI) isolates exhibit in vivo resistance to common antimycobacterial agents, infections caused by this group pose chemotherapeutic problems, often requiring multiple drug regimens, surgery, or both (3, 8). It is therefore important to identify those antimicrobial agents that could be effective in treating diseases caused by this group of organisms. Several members of the fluoroquinolone group of antibiotics have been reported to have antimycobacterial activities (1, 2, 5; Y. Suzuyama, K. HarA, A. Saito, K. Yamaguchi, S. Kohno, and Y. Shigeno, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother. abstr. no. 45, 1986), to be bactericidal at achievable levels in serum (7, 15), and to be effective against intracellular pathogens in animal models (4). Paradoxically, other reports suggest that members of the MAI complex may be quite resistant to this group of antibiotics (9).

In the present study, two new fluoroquinolones, difloxacin and A-56620, are shown to have in vitro activities against MAI organisms. When the susceptibility patterns of isolates from different sources were compared, strains from animal sources were consistently more resistant to the quinolones and cephalosporins tested. Isolates from patients with acquired immunodeficiency syndrome (AIDS) tended to be more resistant than isolates from patients without AIDS, but they were less resistant to the quinolone compounds than were the strains from animals.

Partial data from this study were presented at the 1987 Annual Meeting of the American Society for Microbiology [G. Geddes, S. Byrne, and W. Black, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, U65, p. 137].

**MATERIALS AND METHODS**

**Bacterial strains.** The organisms used in this study were identified as belonging to the MAI complex (12, 13). There were 38 strains from human sources, including 8 from patients with AIDS, isolated from specimens submitted to this laboratory from physicians and other laboratories within British Columbia. A total of 16 strains were isolated from the autopsy material of diseased animals, which included 12 chickens, 2 doves, 1 dog, and 1 pheasant. All cultures were maintained on Lowenstein-Jensen agar slopes (Lowenstein medium base; Difco Laboratories, Detroit, Mich.)

**Antimicrobial agents.** The following antimicrobial reference standard powders were obtained from the indicated sources: difloxacin and A-56620, Abbott Laboratories, Ltd., Chicago, Ill.; cephapirin sodium, Bristol Laboratories of Canada, Ottawa, Ontario; cefazolin, Eli Lilly Canada Inc., Scarborough, Ontario; cefotaxime, Roussel (Canada) Inc., Montreal, Quebec; SK&F 88070 and ceftizoxime, Smith Kline & French Laboratories, Philadelphia, Pa.; and spectinomycin, The Upjohn Co. of Canada, Etobicoke, Ontario. Stock solutions of each drug were prepared according to the instructions of the manufacturers (with the exception of SK&F 88070, which was dissolved in a 0.1 M phosphate buffer at pH 6.8) and filtered through a 0.2-μm-pore-size filter. Drug dilutions were made in distilled water and used immediately.

**Drug susceptibility tests.** All MIC determinations were performed on Middlebrook and Cohn 7H10 agar base medium (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% Middlebrook OADC (oleic acid, albumin, dextrose, catalase) enrichment (Difco). The concentrations of antibiotics used in the agar plates were log₂ dilutions in the range of 64 to 0.25 μg/ml. Medium control plates lacked antibiotic but contained an equivalent amount of the solvent that was used to dissolve each antibiotic powder.

To prepare the inoculum, growth from 1- to 4-week Lowenstein-Jensen slopes were suspended in dilution fluid (0.02% Tween 80, 0.2% bovine serum albumin). After the larger particles were allowed to settle, the suspension was adjusted to the density of a McFarland no. 1 turbidity standard. This was further diluted to 10⁻³ and 10⁻⁴ with distilled water. The inoculum was applied to the surface of the agar plates with a Steers replicating device (10, 11). All plates were incubated at 37°C in 5% CO₂ and examined for

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TABLE 1. Comparison of MICs obtained by the standard 1% proportion method and the Steers replicator method

| Antimicrobial agent | No. of organisms tested | No. that agreed to ± 1 dd\a | No. with b: | PM = RM | RM < PM | RM > PM |
|---------------------|-------------------------|----------------------------|-------------|---------|---------|---------|
| Cefotaxime          | 13                      | 12                         | 4           | 6       | 3       |
| Difloxacin          | 12                      | 11                         | 6           | 1       | 5       |
| A-56620             | 12                      | 10                         | 3           | 5       | 4       |
| Spectinomycin       | 13                      | 12                         | 3           | 5       | 5       |
| Cefoxolin           | 13                      | 10                         | 3           | 6       | 4       |
| SK&F 88070          | 13                      | 10                         | 9           | 4       | 0       |
| Cephalpin           | 13                      | 10                         | 6           | 5       | 2       |
| Cefizoxime          | 13                      | 9                          | 6           | 4       | 3       |
| Total (%)           | 102                     | 84 (82)                    | 40 (39)     | 36 (35) | 26 (25) |

\a dd, Doubling dilution.
\b PM, Standard 1% proportion method; RM, Steers replicator method.

growth after 2 weeks. Test results were considered acceptable if the number of CFU was between 50 and 300 (with an average of 175 CFU) from the 10^{-3} dilution. The MIC was the lowest concentration of antibiotic which inhibited the growth of 99% of the inoculum when compared with the control.

Comparative MIC determinations were performed by using the standard 1% proportional agar dilution method (12) in which the 10^{-3} or 10^{-5} inoculum (approximately 0.1 ml) was placed onto the agar surface, as discrete drops, with a pipette.

Drug stability. The stabilities of the antimicrobial agents in 7H10 agar were determined by an agar diffusion assay. Fresh stock solutions of the agents were prepared and incorporated into 7H10 agar plates. The agar plates were then incubated for 14 days at 37°C in 5% CO2. At various intervals of incubation, cylindrical cores of agar (diameter, 6 mm) were cut through the agar and immediately stored at -70°C.

The amounts of active antimicrobial agents remaining in the agar cores were determined by placing the thawed cores onto the surface of Mueller-Hinton agar plates inoculated with a lawn of Escherichia coli ATCC 25922. After incubation for 24 h at 37°C, the zones of inhibition around the cores were measured. The amounts of active antimicrobial agents remaining in the cores were determined by comparing these zones with a standard curve of the logarithm of the antimicrobial agent concentration (in agar cores) versus zone size for each agent tested.

Statistics. MICs were transformed to log2 values, and the means of these values were compared by the two-sample Student t test. The variances of the samples were tested for homogeneity by the F test. In cases in which these variances were not equal, i.e., the significance value of the F test was less than 0.10, the Satterthwaite approximation for degrees of freedom was used (14).

RESULTS

Comparison of inoculation techniques in agar dilution method for MIC determinations. A comparison of the MIC results obtained with a Steers replicating device and by the standard 1% proportion method of inoculation are presented in Table 1. The two methods correlated within one doubling dilution 82% of the time. Of the discrepant results, a greater number had lower MICs by the Steers replicator method.

Both methods of inoculation were about equally reproducible. Of 48 repeated results, 45 (94%) obtained by the 1% proportion method were within one doubling dilution, and 53 of 55 (96%) comparisons from the Steers replicator method were within this range. Because of the relative ease with which a large number of organisms can be tested when the Steers replicating device is used, we proceeded to conduct our study using this method.

Antimicrobial stability. The half-lives of the antimicrobial agents in 7H10 agar were determined. No reduction of activity was detected for either of the two quinolone compounds (A-56620 and difloxacin) after 14 days of incubation. The other agents had the following approximate half-lives: spectinomycin, 33 days; cefazolin, 17 days; cefizoxime, 11 days; SK&F 88070, 9 days; cefotaxime, 5 days; and cephalpin, less than 1 day.

MIC determinations. The distributions of the MICs of the antibiotics tested against isolates from humans without AIDS are presented in Table 2. The quinolones difloxacin and A-56620 were the most active agents in vitro, each of which had MICs for 50% of strains tested (MIC50s) of 2 μg/ml. All other drugs had MIC50s of at least 8 μg/ml. Isolates from patients with AIDS were significantly more resistant to all agents tested than were MAI isolates from other patients (Table 3).

Comparison of the drug susceptibility patterns of strains from animal sources. Isolates from humans and animals differed in their apparent susceptibilities to the agents tested. Strains from animals were significantly more resistant than were the isolates from humans without AIDS for each of the agents tested (Table 4). However, compared with isolates from patients with AIDS, strains from animals were significantly more resistant to only the quinolone compounds (Table 5).

TABLE 2. Distribution of MICs for 30 MAI isolates from humans without AIDS against eight antimicrobial agents

| Antimicrobial agent | No. of organisms for which MICs (μg/ml) were: |
|---------------------|---------------------------------------------|
|                     | 0.5 | 1  | 2  | 4  | 8  | 16 | 32 | 64 | >64 |
| A-56620             | 1   | 7  | 10a| 7  | 1  | 3  | 1  |    |    |
| Difloxacin          | 1   | 3  | 11a| 9  | 5  | 1  |    |    |    |
| Cefazolin           | 1   | 4  | 2  | 8  | 3  | 3  | 6  | 3  |    |
| Cefotaxime          | 1   | 2  | 3  | 6  | 3  | 4  | 7  |    |    |
| Cefizoxime          | 1   | 2  | 1  | 4  | 4  | 4  | 5  | 8  |    |
| Cephalpin           | 4   | 5  | 4  |    | 6  | 6  | 2  |    |    |
| SK&F 88070          | 1   | 2  | 4  | 1  | 2  | 4  | 7  | 7  |    |
| Spectinomycin       | 1   | 3  | 7  | 4  | 8  |    |    |    |    |

\a MICs for these organisms were also the MIC50s.
TABLE 4. Comparisons of antimicrobial susceptibilities of isolates from humans without AIDS and animals

| Antimicrobial agent | MIC<sub>50</sub> (µg/ml) for isolates from: | Difference of mean log<sub>2</sub> MICs | P value |
|---------------------|------------------------------------------|-------------------------------------|---------|
|                     | Humans (n = 30) | Animals (n = 16) |                     |                              |
| A-56620             | 2 >64          | 5.1             | <0.0001             |
| Difloxacin          | 2 64           | 4.0             | <0.0001             |
| Cefazolin           | 8 64           | 1.9             | <0.0001             |
| Cefotaxime          | 32 >64         | 2.4             | <0.0001             |
| Cefotizoxine        | 32 >64         | 2.2             | <0.0001             |
| Cephapirin          | 16 64          | 1.9             | <0.0001             |
| SK&F 88070          | 32 >64         | 2.4             | <0.0001             |
| Spectinomycin       | 32 64          | 0.8             | 0.014               |

DISCUSSION

Because of the failure of current drug therapy to control MAI infections, it is important to identify new agents that are efficacious in the treatment of MAI (5). Of the antimicrobial agents tested, the two arylfluoroquinolones difloxacin and A-56620 proved to be the most effective in vitro against isolates from humans. The other antimicrobial agents tested, if at all effective, were variable in their in vitro effects on the MAI group.

Because of the slow growth of MAI, it was necessary to determine the extent of drug inactivation in 7H110 agar. Four of the antimicrobial agents tested (A-56620, difloxacin, cefazolin, and spectinomycin) had half-lives greater than the 2-week period of incubation, indicating no significant degradation. Cefotaxime and cephapirin had half-lives of less than 1 week. For these, caution should be exercised in the interpretation of results, since a pattern of resistance may be the result of antimicrobial degradation to a concentration below that which is bacteriostatic to the strain being tested.

Since MAI organisms cause disease in a wide variety of animals, we were interested to determine the degree of similarity between isolates from animals and those from humans. The results we presented here indicate that isolates from animals are different from those that cause disease in humans. Isolates from patients without AIDS were significantly more susceptible to all the agents tested compared with those from animal sources. Isolates from patients with AIDS, although similar in their susceptibilities to the cephalosporins and spectinomycin, were significantly more susceptible to the two quinolone agents than were the isolates from animals. These differences support previous observations that MAI isolates from patients with AIDS differ from those that infect other patients (6) and suggest that animals are an unlikely source of MAI organisms that cause human infections in British Columbia.

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