In Vitro Susceptibility of *Mycobacterium marinum* to Eight Antimicrobial Agents

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The in vitro susceptibilities of 16 *Mycobacterium marinum* strains to eight antimicrobial agents were determined by the agar dilution technique. The most active drugs were amikacin and kanamycin. Tetracycline, doxycycline, and minocycline were inhibitory, predominantly at concentrations slightly below the expected blood and tissue levels. Trimethoprim-sulfamethoxazole and erythromycin demonstrated activity only at concentrations greater than those usually attained in serum and tissues. Gentamicin was relatively inactive.

*Mycobacterium marinum* has been recognized as a cause of human soft-tissue disease, the so-called “swimming pool granuloma,” since 1951 (15). Now, other varieties of skin and subcutaneous involvement, including “fish tank granuloma,” are more commonly seen (21). Although spontaneous healing usually occurs, disability may continue for many months. In addition to local therapy, several conventional antituberculosis drugs and other antimicrobial agents have been used with success (21), although evidence for the in vitro effectiveness of many of these agents often was lacking. This study was designed to determine the in vitro susceptibility of *M. marinum* to several drugs which have been said to be effective, or which have shown promise in other mycobacterial infections. These included amikacin, gentamicin, kanamycin, tetracycline, doxycycline, minocycline, trimethoprim-sulfamethoxazole (TMP-S), and erythromycin.

**MATERIALS AND METHODS**

Sixteen strains of *M. marinum* were obtained from the mycobacteriology laboratory of Cleveland Metropolitan General Hospital. These strains included 11 recent isolates from human soft-tissue infections (three were provided by William Hanke of the Cleveland Clinic) and five laboratory strains from the Mycobacterial Culture Collection formerly at the Trudeau Institute, Saranac Lake, N. Y., or from Werner Schaefer (one strain was the original Arnon fish strain [1]).

Susceptibilities were determined by the agar dilution method, using serial twofold dilutions of drugs in Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) contained in quadrant plates. In a preliminary experiment with three strains using the disk diffusion method, most of the drugs showed greater activity in Mueller-Hinton agar than in Middlebrook 7H10 agar (Difco Laboratories, Detroit, Mich.). Drug concentrations ranged from 0.3 to 10 μg of amikacin, gentamicin, and kanamycin per ml; 0.6 to 20 μg of doxycycline, minocycline, and TMP-S per ml; 3.1 to 100 μg of tetracycline per ml; and 1.2 to 40 μg of erythromycin per ml. The test inoculum was prepared in Middlebrook 7H9 broth from an actively growing culture and adjusted visually to turbidity of the no. 1 McFarland standard. Serial 10-fold dilutions of the resulting suspension were made, and 1 drop each of the 1:10, 1:100, and 1:1,000 suspensions was deposited on each quadrant of the test plates and on a control plate without drugs. Only nine strains were evaluated with amikacin and gentamicin.

The plates were incubated at 30°C for 14 days. The minimal inhibitory concentration was defined as the lowest concentration of each agent showing complete inhibition of growth as viewed under the dissecting microscope, discarding those inoculum sizes that yielded either confluent growth or less than 50 colonies on the control quadrants.

**RESULTS**

The minimal inhibitory concentrations are depicted in Fig. 1. Amikacin and kanamycin produced the greatest inhibition with 89 and 62% of strains, respectively, inhibited at 2.5 μg/ml, and 100 and 94%, respectively, inhibited at 5 μg/ml. Gentamicin, however, inhibited only 44% of strains at 10 μg/ml, the highest concentration tested.

Tetracycline, doxycycline, and minocycline all showed similar activity, (minimal inhibitory concentrations ranging from 2.5 to 20 μg/ml) requiring approximately 4 to 7 μg/ml to inhibit 50% of the strains. TMP-S also showed activity in this range, but the endpoint was sometimes difficult to determine because of persistent growth of tiny colonies. Erythromycin was slightly less inhibitory than the tetracyclines and TMP-S.

**DISCUSSION**

The drugs which have been reported to be clinically effective, either as single agents or in combination, in the treatment of *M. marinum*...
infections include clofazimine (2), rifampin (22), ethambutol (22), kanamycin (17), TMP-S (3, 4, 11, 19), tetracycline (9, 12), and minocycline (5, 13, 14). Isoniazid and streptomycin, two widely used drugs for mycobacterial infections, showed only slight activity against *M. marinum* strains, with reported minimal inhibitory concentrations of 20 μg/ml (22) and 2 to 10 μg/ml (6, 22), respectively. The minimal inhibitory concentrations of rifampin and ethambutol are 1.0 and 2.0 μg/ml, respectively, and these drugs are usually effective clinically (22).

The present study documents the in vitro effectiveness of kanamycin and amikacin, which inhibited all of the test strains at concentrations well within obtainable serum levels. Gentamicin, however, was much less effective.

Although tetracyclines were clinically effective (5, 9, 12-14), two studies noted in vitro resistance of *M. marinum* to tetracycline at 100 μg/ml (12) and 25 to 50 μg/ml (9). Because these concentrations greatly exceeded the usual achievable blood levels (3 to 6 μg/ml), the therapeutic effect was considered by one author to be on a basis other than antibacterial activity (16). In the present investigation, it was found that the three tetracyclines inhibited 50% of the strains at levels that might be obtained in the tissues. These results are similar to those of a previous study in which 32 strains were tested against minocycline and doxycycline using the agar dilution technique and Middlebrook 7H11 agar (18).

TMP-S was effective clinically in several studies (3, 4, 11, 19), and *M. marinum* strains have been reported to be "susceptible in vitro" without details of methodology (3, 4, 7, 8). In the present study, TMP-S inhibited 100, 88, and 31% of strains at 20, 10, and 5 μg of trimethoprim per ml, respectively. Since serum trimethoprim levels after the usual doses of TMP-S are generally 3 μg/ml (10), the relationship between these in vitro data and reported clinical effectiveness is uncertain.

Erythromycin alone or in combination with other agents yielded equivocal results in the treatment of *M. marinum* infections (6, 20). In the present study, the minimal inhibitory concentrations were found to be several times higher than the usual achievable concentrations in serum.

As far as we could determine, the 16 cultures reported in this study represented 16 different strains. Five others sent as proficiency test samples by the College of American Pathologists and the Center for Disease Control were duplicates of two stock strains, and these were included in the data used to construct Fig. 1. Omitting these five strains did not change the curves appreciably.

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**LITERATURE CITED**