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Susceptibilities of *Mycobacterium marinum* to Gatifloxacin, Gemifloxacin, Levofloxacin, Linezolid, Moxifloxacin, Telithromycin, and Quinupristin-Dalfopristin (Synercid) Compared to Its Susceptibilities to Reference Macrolides and Quinolones

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The susceptibility pattern of *Mycobacterium marinum* was determined. Quinupristin-dalfopristin and telithromycin were less active than clarithromycin. Linezolid showed good antimicrobial activity at clinically achievable concentrations. Gatifloxacin, levofloxacin, and moxifloxacin displayed activities similar to those of ciprofloxacin. Gemifloxacin was less active. The Etest method showed variable agreement with the reference method.

Mycobacterium marinum was recognized as a human pathogen in 1959 (13). Infection occurs when traumatized skin is exposed to contaminated water and is usually localized to the skin of the upper extremity. The organism can also cause deep-tissue infection and, rarely, disseminated disease (4, 5, 6). The incubation period has been estimated to between 2 and 4 weeks, and the infection usually resolves spontaneously after 6 to 30 months. Since infection with *M. marinum* is quite rare and the organism is resistant to many conventional antimicrobial agents, optimal treatment has yet to be established. The aim of this study was to determine the susceptibility of *M. marinum* to new antimicrobial agents. Furthermore, the Etest method was evaluated.

Of the strains tested, 43 were clinical isolates collected in Sweden from 1989 to 2001. *M. marinum* ATCC 2275 was also included as a reference strain. Ciprofloxacin and moxifloxacin were from Bayer, Wuppertal, Germany; clarithromycin was from Abbott, Queenborough, United Kingdom; erythromycin was from Astra, Södertälje, Sweden; gatifloxacin was from Grünenthal, Stolberg, Germany; gemifloxacin was from SmithKline Beecham, Munich, Germany; linezolid was from Pharmacia & Upjohn, Kalamazoo, Mich.; and levofloxacin, quinupristin-dalfopristin, and telithromycin were from Aventis Pharma, Vitry sur Seine, France.

The MICs of the drugs were determined by using Mueller-Hinton agar supplemented with 5% OADC (oleic acid, albumin, dextrose, and catalase) (1). A multipoint inoculator was used to deliver approximately 10^4 to 10^5 CFU per spot to agar dilution plates containing antimicrobial agents in twofold dilutions. All the plates were incubated at 30°C for 7 days. The MICs for the *M. marinum* strains were defined as the lowest concentration allowing no visible growth at the spot of inoculation on the agar dilution plates. The MICs for *Staphylococcus aureus* ATCC 21212 and *Enterococcus faecalis* ATCC 21213 were determined after 24 h and shown to be within reference values for all antibiotics. Etest strips were obtained from Biodisk (Solna, Sweden). The correlation of the results determined by the Etest method and the agar dilution method, used as a reference, was determined on the basis of the agreement, which was defined as the percentage of clinical isolates for which the MIC was the same within ± 1 $\log_2$ dilution.

The MICs of 10 antimicrobial agents determined by the agar dilution method are presented in Table 1. The MICs of clarithromycin (MIC at which 50% of the isolates tested were inhibited [MIC_{50}], 1 μg/ml; MIC_{90}, 1 μg/ml) were lower than those for erythromycin (MIC_{50}, 16 μg/ml; MIC_{90}, >32 μg/ml). The ketolide telithromycin (MIC_{50}, >32 μg/ml; MIC_{90}, >32 μg/ml) and the streptogramin quinupristin-dalfopristin (MIC_{50}, >32 μg/ml; MIC_{90}, >32 μg/ml) had higher MICs than the reference macrolide compounds. The oxazolidone linezolid (MIC_{50}, 2 μg/ml; MIC_{90}, 2 μg/ml) was found to have the second lowest MICs of all the antimicrobial agents tested. The fluoroquinolones gatifloxacin and moxifloxacin had the same MICs (MIC_{50}, 4 μg/ml; MIC_{90}, 8 μg/ml) as the reference quinolone ciprofloxacin. The MICs of levofloxacin (MIC_{50}, 8 μg/ml; MIC_{90}, 16 μg/ml) and gemifloxacin (MIC_{50}, 16 μg/ml; MIC_{90}, >32 μg/ml) were higher.

The Etest method was compared to the reference agar dilution method. The percentages of agreement between the MICs determined by the agar dilution and Etest methods are presented in Table 2. The percentages of agreement were higher for gemifloxacin (92%), moxifloxacin (80%), clarithromycin (76%), and linezolid (76%) than for ciprofloxacin (57%) and levofloxacin (8%).

Regarding the macrolides, clarithromycin is recommended by the American Thoracic Society and has, in addition to surgical excision, been used successfully for the treatment of clinical *M. marinum* infections (2, 18). We compared the activities of telithromycin and quinupristin-dalfopristin with those of clarithromycin and erythromycin. The tested *M. marinum* strains were found to be far more susceptible to clarithromycin than to any other macrolide. This result is in agreement with previous findings regarding *M. marinum* (C. Truffot-

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TABLE 1. MICs of 10 antimicrobial agents for 43 clinical isolates of *M. marinum*, determined with the agar dilution method

Antimicrobial agent	MIC (μg/ml)		
	50%	90%	Range
Clarithromycin	1	1	0.125–1
Erythromycin	16	>32	8–>32
Linezolid	2	2	0.5–4
Quinupristin-dalfopristin	>32	>32	8–>32
Telithromycin	>32	>32	>32
Ciprofloxacin	4	8	0.5–8
Gatifloxacin	4	8	0.5–8
Gemifloxacin	16	>32	4–>32
Levofloxacin	8	16	2–16
Moxifloxacin	4	8	0.5–8

Pernot, N. Lounis, F. Chantot, and J. Grosset, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F167, p. 142, 1995) and other mycobacteria (1, 7, 14). Oxazolidones have been reported to be effective against mycobacteria and also for treating complicated skin and soft tissue infections, facts that prompted us to select it for susceptibility testing against *M. marinum* (16, 19). In our study, linezolid was the second most active antimicrobial agent after clarithromycin, with antimicrobial activity at concentrations below those in serum and tissue at conventional dosages. The pharmacological properties of linezolid (3) make it an interesting candidate for clinical use.

Fluoroquinolones have been reported to be active against *M. marinum* as well as other mycobacteria (1, 7,15, 17). We found that none of the new fluoroquinolones tested was more active against *M. marinum* than ciprofloxacin. In decreasing order of activity, the fluoroquinolones were ranked as follows: ciprofloxacin = gatifloxacin = moxifloxacin > levofloxacin > gemifloxacin. The MICs determined by us were in agreement with those determined in some previous studies, although somewhat lower MICs have been reported from other laboratories (1, 9, 20). Moxifloxacin has been reported to have lower MICs than ciprofloxacin against *M. marinum* (1) and against other mycobacteria (11). Gatifloxacin has been reported to be more active than ciprofloxacin against *M. tuberculosis* but to be ineffective against *M. avium* (10). Gemifloxacin, on the other hand, has been reported to be less active than ciprofloxacin against *M. tuberculosis* (15). It is unlikely that the quinolones tested will be useful for the treatment of *M. marinum* infections because the MICs are at or close to the peak concentrations in serum.

TABLE 2. Correlation of MICs determined by Etest and agar dilution (reference) methods

Antimicrobial agent	No. of Etest MICs within the indicated $\log_2$ dilution of agar dilution MICs							% Agreement[a]
	>−2	−2	−1	0	1	2	>2	
Clarithromycin	4	2	7	13	8	3	0	76
Erythromycin	0	0	0	35	8	0	0	100
Linezolid	0	6	7	18	4	3	0	76
Quinupristin-dalfopristin	0	0	0	40	0	0	0	100
Ciprofloxacin	3	6	6	9	8	1	7	57
Gemifloxacin	1	2	0	5	31	0	0	92
Levofloxacin	0	3	1	1	1	25	7	8
Moxifloxacin	3	1	7	19	7	4	0	80

[a] Percentage of clinical isolates for which the MIC was the same within ± 1 $\log_2$ dilution.

The Etest method has been described as having acceptable results for rapidly growing mycobacteria (12) but more divergent results for *M. marinum* compared to agar dilution methods (1, 8, 20). We found that resistance to erythromycin and quinupristin-dalfopristin always was detected by the Etest method; relatively good agreement was obtained between the Etest and agar dilution methods for gemifloxacin and moxifloxacin, but a lower correlation was obtained for ciprofloxacin and levofloxacin. A major problem that we encountered was trailing endpoints and gradually decreased growth, giving indistinct inhibition zones and thus results that were difficult to interpret, especially for bacteriostatic drugs. This fact also forced us to exclude some strains for which MICs could not be determined. Thus, we agree with Aubry et al. (1) that the agar dilution method is more accurate than the Etest method for antibiotic susceptibility testing of *M. marinum*, and we emphasize the lack of suitability of the Etest method for *M. marinum*.

In conclusion, we described the pattern of susceptibility of *M. marinum* to 10 different antimicrobial agents, some of which were tested for the first time against this species. Of all the new drugs tested, only linezolid could be considered of interest for the treatment of *M. marinum* infections in humans on the basis of in vitro activity and pharmacokinetics. We hope that the results from our investigation will be helpful for clinical trials that eventually could lead to more efficient treatment of patients infected with *M. marinum*.

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