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In Vitro Susceptibilities of Rapidly Growing Mycobacteria to Newer Antimicrobial Agents

NANCY KHARDORI,[1,2]* HIEU NGUYEN,[2] BEVERLY ROSENBAUM,[2] KENNETH ROLSTON,[2] AND GERALD P. BODEY[2]

Division of Infectious Diseases, Department of Internal Medicine, Southern Illinois University School of Medicine, Springfield, Illinois 62794,[1] and Section of Infectious Diseases, Department of Medical Specialties, University of Texas MD Anderson Cancer Center, Houston, Texas 77030[2]

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The in vitro antimicrobial susceptibilities of 42 isolates of rapidly growing mycobacteria (*Mycobacterium fortuitum*, *M. chelonae*, and *Mycobacterium* species [other than *M. fortuitum* and *M. chelonae*]) to nine quinolones, including newer agents, two new aminoglycosides, and an aminocyclitol (trospectomycin) were determined by a broth microdilution method. The new quinolones, PD 117596, PD 127391, and PD 117558, showed excellent in vitro activities against *M. fortuitum* (MICs for 90% of isolates [MIC_{90}s], 0.06, 0.06, and 0.12 μg/ml, respectively). The MIC_{90} of ciprofloxacin for *M. fortuitum* was 0.5 μg/ml. Only 14 to 28% of isolates of *M. chelonae* were susceptible to various quinolones. Most isolates of all three species were susceptible to the new aminoglycosides SCH 21420 and SCH 22591. The MIC_{90}s of trospectomycin were 8 μg/ml for *M. chelonae*, 32 μg/ml for *Mycobacterium* species, and >64 μg/ml for *M. fortuitum*.

The rapidly growing mycobacteria represent a heterogenous group of environmental mycobacteria. *Mycobacterium fortuitum* and *M. chelonae* are the species most often associated with clinical disease. These organisms produce pulmonary infection only rarely but are responsible for a large number of primary skin and soft tissue infections (39). Infections caused by these organisms are usually localized, but disseminated disease has been reported (1, 6, 13, 20–22, 32, 33, 36, 39). These organisms are also resistant to the standard antituberculous agents. Recent in vitro studies have revealed the activity of a number of antibacterial agents against *M. fortuitum* and *M. chelonae*. In an effort to identify potentially useful compounds, we determined the in vitro susceptibilities of 20 isolates of *M. fortuitum*, 14 isolates of *M. chelonae*, and 8 isolates of *Mycobacterium* species (other than *M. fortuitum* and *M. chelonae*) to 9 quinolones, including seven new agents (2–5, 10–12, 15, 17–19, 22–24, 30, 31, 36, 40, 41), two new aminoglycosides (7, 16, 29), and trospectomycin (an analog of spectinomycin). Species identification was done by high-pressure liquid chromatography.

The antimicrobial agents used in this study and their sources were ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.); norfloxacin (Merck Sharp & Dohme, Rahway, N.J.); PD 117596, PD 127391, and PD 117558 (Warner Lambert Pharmaceutical Research, Ann Arbor, Mich.); S25930 and S25932 (Riker Laboratories, St. Paul, Minn.); A56620 (Abbott Laboratories, North Chicago, Ill.); amifloxacin (Sterling-Winthrop Research Institute, Rensselaer, N.Y.); SCH 21420 and SCH 22591 (Schering-Plough Research, Kenilworth, N.J.); and trospectomycin (The Upjohn Co., Kalamazoo, Mich.).

The isolates were maintained on slants of Lowenstein-Jensen medium prior to use. The in vitro activities of various agents against rapidly growing mycobacteria were determined by a previously described broth microdilution method (33). Serial twofold dilutions of antimicrobial agent solutions were added to Mueller-Hinton broth by use of the MIC 2000 system (Dynatech Laboratories, Inc., Alexandria, Va.). The organisms were subcultured on Trypticase soy sheep blood agar plates and incubated at 35°C for 2 to 4 days. Three to five colonies were inoculated into 5 ml of Mueller-Hinton broth supplemented with 0.02% Tween 80. The cultures were incubated at 35°C for 1 to 3 days on a shaking water bath. The suspensions were standardized to a turbidity equal to a 0.5 McFarland barium sulfate standard. An inoculum of 10 μl was added to each well, yielding a final concentration of 5×10^5 to 1×10^6 CFU/ml. Inoculated plates were sealed inside plastic bags and incubated at 35°C. The MICs were read at 3 days. CFU in suspensions with a turbidity equivalent to a 0.5 McFarland standard were determined by subculturing on Trypticase soy sheep blood agar plates. The viable counts ranged from 5×10^6 to 1×10^8 CFU/ml.

Any deterioration of antimicrobial activity during prolonged incubation was measured by determination of the antimicrobial susceptibilities of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 in freshly prepared trays and trays incubated for 1 and 3 days.

As shown in Table 1, 93 to 100% isolates of *M. fortuitum* were inhibited by all the quinolones, except for S25930 and S25932. The MICs for 90% of isolates of the new aminoglycosides SCH 21420 and SCH 22591 for *M. fortuitum* were 16 and 2 μg/ml, respectively. In comparison, only 14 to 28% of isolates of *M. chelonae* were susceptible to the various quinolones tested, whereas all isolates were susceptible to the aminoglycosides and trospectomycin. The in vitro susceptibilities of *Mycobacterium* species were generally similar to those of *M. fortuitum*.

The rapidly growing mycobacteria vary widely in their in vitro susceptibilities to the currently available antimicrobial agents (8, 9, 25–28, 34, 35, 37). Amikacin is the most predictably active agent, followed by other aminoglycosides, cefoxitin, doxycycline, and rifampin. Some isolates may be susceptible to ethambutol, sulfonamides, or erythromycin. Treatment with multiple agents is preferable because of a

* Corresponding author. Mailing address: Division of Infectious Diseases, Department of Internal Medicine, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794-9230. Phone: (217) 782-0181. Fax: (217) 788-5504.

TABLE 1. In vitro activities of newer antimicrobial agents against rapidly growing mycobacteria

Organism (no. tested) and antimicrobial agent	MIC (μg/ml)[a]			% Susceptible
	Range	50%	90%	
M. fortuitum (20)				
Quinolones				
Ciprofloxacin	<0.03–1	0.06	0.5	100
Norfloxacin	0.25–8	1	8	93
PD 117596	<0.0075–0.12	0.03	0.06	100
PD 127391	<0.0075–0.12	0.03	0.06	100
PD 117558	<0.03–0.25	0.12	0.12	100
S25930	1–32	4	16	50
S25932	1–32	4	16	57
Amifloxacin	0.25–8	1	4	100
A56620	0.06–2	0.25	2	100
Aminoglycosides and an aminocyclitol				
SCH 21420	0.5–32	8	16	93
SCH 22591	0.12–8	0.5	2	93
Trospectomycin	2–>64	16	>64	43
M. chelonae (14)				
Quinolones				
Ciprofloxacin	0.12–64	4	64	28
Norfloxacin	1–>64	64	>64	14
PD 117596	0.5–>16	8	>16	28
PD 127391	0.015–>16	8	>16	28
PD 117558	<0.03–0.25	16	64	28
S25930	0.5–>64	>64	>64	14
S25932	1–>64	>64	>64	14
Amifloxacin	1–>64	>64	>64	14
A56620	0.12–>64	>64	>64	14
Aminoglycosides and an aminocyclitol				
SCH 21420	1–8	2	8	100
SCH 22591	0.5–4	0.5	4	100
Trospectomycin	2–8	4	8	100
Mycobacterium species (8)				
Quinolones				
Ciprofloxacin	<0.03–1	0.25	1	100
Norfloxacin	<0.03–8	1	8	75
PD 117596	0.0075–0.12	0.03	0.12	100
PD 127391	0.015–0.06	0.03	0.06	100
PD 117558	<0.03–0.5	0.12	0.5	100
S25930	2–8	4	8	62
S25932	1–8	4	8	62
Amifloxacin	2–8	2	8	100
A56620	0.25–2	1	2	100
Aminoglycosides and an aminocyclitol				
SCH 21420	<0.03–1	0.12	1	100
SCH 22591	<0.03–1	0.12	4	100
Trospectomycin	0.12–32	0.5	32	87

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

high rate of relapse and the emergence of drug resistance (9, 25, 35). Imipenem-cilastatin, amoxicillin-clavulanate, and ciprofloxacin are among the newer agents with in vitro activities against some isolates of rapidly growing mycobacteria (39). Wallace et al. (38), reported on the susceptibilities of 548 clinical isolates of rapidly growing mycobacteria to ciprofloxacin. The 170 isolates of *M. fortuitum* biovar fortuitum were the most susceptible (MIC for 90% of isolates, 0.125 μg/ml), and the 62 *M. chelonae* isolates were the most resistant. Mutational frequencies with ciprofloxacin were relatively high for *M. fortuitum* (10^{-5} to 10^{-7}). The MICs for single-step mutants were similar to those for resistant isolates. These data demonstrate the need for combination therapy of infections caused by rapidly growing mycobacteria, even when newer agents are used. N-substituted analogs of ciprofloxacin (*N*-methyl ciprofloxacin and *N*-ethyl ciprofloxacin) showed significantly improved activities against *M. tuberculosis*, but the activities against *M. fortuitum* and *M. chelonae* were similar to those of ciprofloxacin (14). Our results on the in vitro activities of ciprofloxacin against *M. fortuitum* and *M. chelonae* are similar to those published earlier (14, 38). The data presented here also indicate that a

number of newer quinolones (PD 117596, PD 12739, and PD 117558), amifloxacin, and A56620 have potent inhibitory activities against *M. fortuitum* and *Mycobacterium* species but not against *M. chelonae*. The activities of trospectomycin for the three groups of organisms were variable. The newer aminoglycosides SCH 21420 and SCH 22591 showed in vitro activities against most isolates from all three groups.

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