ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1997, p. 1475–1481 Vol. 41, No. 7
0066-4804/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Comparative Activity of Trovafloxacin, Alone and in Combination with Other Agents, against Gram-Negative Nonfermentative Rods

MELISSA A. VISALLI,[1] SARALEE BAJAKSOUZIAN,[2] MICHAEL R. JACOBS,[2] AND PETER C. APPELBAUM[1]*

Departments of Pathology (Clinical Microbiology), Hershey Medical Center, Hershey, Pennsylvania 17033,[1] and Case Western Reserve University, Cleveland, Ohio 44106[2]

Received 29 January 1997/Returned for modification 14 April 1997/Accepted 23 April 1997

In the first part of this study, agar dilution MICs were used to test the activities of trovafloxacin, ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, clinafloxacin, ceftazidime, and imipenem against 458 gram-negative nonfermenters. The overall respective MICs at which 50% of isolates are inhibited (MIC_{50}s) and MIC_{90}s were as follows: trovafloxacin, 1.0 and 16.0 μg/ml; ciprofloxacin, 2.0 and 16.0 μg/ml; ofloxacin, 2.0 and 32.0 μg/ml; levofloxacin, 1.0 and 16.0 μg/ml; sparfloxacin, 1.0 and 16.0 μg/ml; clinafloxacin, 0.5 and 4.0 μg/ml; ceftazidime, 8.0 and 128.0 μg/ml; imipenem, 2.0 and 256.0 μg/ml. Clinafloxacin was the most active of all the quinolones tested. The MIC_{90}s of trovafloxacin were ≤4.0 μg/ml for *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Flavobacterium odoratum*, and *Chryseobacterium meningosepticum*; trovafloxacin MIC_{90}s were ≤2.0 μg/ml for *Moraxella* spp., *Pseudomonas stutzeri*, and *Chryseobacterium indologenes-C. gleum*. Of the other quinolones tested, the MICs of sparfloxacin and levofloxacin were lower than those of ciprofloxacin and ofloxacin. High ceftazidime MICs (≥32.0 μg/ml) were observed for all nonfermentative species tested. Although for the majority of strains tested imipenem MICs were ≤8.0 μg/ml, high imipenem MICs were observed for many species, especially *S. maltophilia*, *Burkholderia cepacia*, *F. odoratum*, and *Chryseobacterium meningosepticum*. For *Alcaligenes xylosoxidans* strains, the MICs of all compounds were generally a few dilutions lower than those for *Alcaligenes faecalis-A. odorans*. Time-kill studies with five strains revealed that trovafloxacin and all quinolones yielded more rapid time-kill kinetics than ceftazidime and imipenem. Synergy testing by checkerboard titrations of 286 strains with trovafloxacin combined with ceftazidime, amikacin, and imipenem revealed fractional inhibitory concentration (FIC) indices in the range indicating synergism (≤0.5) for 81, 41, and 40 strains, respectively, and FIC indices indicating additivity or indifference (>0.5 to 4.0) for 205, 245, and 246 strains, respectively. No FIC indices indicating antagonism (>4.0) were observed. Synergy between trovafloxacin and ceftazidime was found for 32 of 36 *S. maltophilia* strains. Time-kill studies with 20 strains showed that for most strains for which FIC indices were in the range indicating additivity or indifference, FIC indices indicated synergy by the time-kill method. Synergy was particularly noticeable for *S. maltophilia* strains with combinations of ceftazidime and trovafloxacin.

Gram-negative nonfermentative rods are increasingly implicated as causative agents in human disease. The organisms are acquired as a result of contact with environmental strains as well as through nosocomial transmission (1–4, 18, 21, 38, 42, 43). Although *Pseudomonas aeruginosa* is the nonfermenter most commonly encountered clinically, other gram-negative nonfermentative rods are being recovered from debilitated or immunosuppressed hosts with increasing frequency (1–4, 18, 21, 38, 42, 43). The antimicrobial susceptibility patterns of the nonfermenters differ from those of the members of the family *Enterobacteriaceae* in many respects, and among the nonfermenters, many groups have susceptibility spectra which differ from the spectrum for *P. aeruginosa*. The unpredictability and breadth of drug resistance of many nonfermenters and the development of new antimicrobial agents with wider spectra of activity against these organisms make in vitro susceptibility testing a major component of rational therapy (1–4, 18, 21, 38, 42, 43).

Trovafloxacin is a new broad-spectrum naphthyridone with activity against a wide variety of gram-positive and -negative aerobic and anaerobic bacteria (10, 20, 37). This study was divided into two parts. (i) We first tested the activity of trovafloxacin and compared it with the activities of other quinolone and nonquinolone agents against 458 nonfermenters by the agar dilution method. Five strains were also examined by the time-kill method. (ii) In the second part of the study, we tested whether the activity of the combination of trovafloxacin with ceftazidime, amikacin, and imipenem was synergistic against 286 nonfermenters by the checkerboard titration method and 20 strains by the time-kill method.

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, P.O. Box 850, Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953. E-mail: pappelba@psuhmc.hmc.psu.edu.

MATERIALS AND METHODS

Bacteria. The organisms tested in this study were all clinical isolates collected within the past 7 years from Hershey Medical Center, University Hospitals of Cleveland, the Cleveland Clinic, and Hôpital St. Louis, Paris, France. Data for 50% of strains, especially strains of *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, and *Acinetobacter* genospecies, were not included in a previous report (38) from our laboratory; the *P. aeruginosa* strains tested in the present study were also not included in that study (38). Strains were all identified by conventional methods (18, 42). For the purposes of this study, *Pseudomonas fluorescens* and *Pseudomonas putida* strains and *Chryseobacterium indologenes-C. gleum* were not differentiated. Because of the complicated nature of current taxonomy (42), *Acinetobacter* strains were divided into *Acinetobacter baumannii* strains and non-*Acinetobacter baumannii* strains by gas-liquid chromatographic analysis (MIDI, Newark, Del.). Organisms were frozen at −70°C in double-strength litmus milk prior to testing. Purity was checked throughout the study by Gram staining and examination of colonial morphology.

Agar dilution MIC determination. MICs for 458 strains were determined by the agar dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (27) by using cation-adjusted Mueller-Hinton

TABLE 1. Agar dilution MICs of agents for 458 gram-negative nonfermentative rods

Species and agent (no. of isolates tested)	MIC (μg/ml)		
	Range	50%	90%
Pseudomonas aeruginosa (89)			
Trovafloxacin	0.06–16.0	0.5	2.0
Ciprofloxacin	0.06–16.0	0.25	4.0
Ofloxacin	0.5–64.0	2.0	4.0
Levofloxacin	0.125–32.0	1.0	2.0
Sparfloxacin	0.25–32.0	1.0	2.0
Clinafloxacin	0.06–4.0	0.25	0.5
Ceftazidime	1.0–>256.0	4.0	64.0
Imipenem	0.25–256.0	2.0	32.0
Pseudomonas fluorescens-Pseudomonas putida (13)			
Trovafloxacin	0.25–16.0	1.0	8.0
Ciprofloxacin	0.06–4.0	0.25	4.0
Ofloxacin	0.25–32.0	2.0	16.0
Levofloxacin	0.125–16.0	1.0	8.0
Sparfloxacin	0.125–32.0	1.0	8.0
Clinafloxacin	0.016–4.0	0.25	2.0
Ceftazidime	2.0–128.0	4.0	64.0
Imipenem	0.25–256.0	2.0	256.0
Burkholderia cepacia (49)			
Trovafloxacin	0.125–128.0	8.0	32.0
Ciprofloxacin	0.25–>256.0	8.0	32.0
Ofloxacin	2.0–256.0	16.0	64.0
Levofloxacin	0.5–128.0	8.0	16.0
Sparfloxacin	0.125–128.0	4.0	32.0
Clinafloxacin	0.125–32.0	2.0	8.0
Ceftazidime	1.0–128.0	8.0	32.0
Imipenem	0.125–256.0	16.0	256.0
Stenotrophomonas maltophilia (82)			
Trovafloxacin	0.06–16.0	1.0	2.0
Ciprofloxacin	0.25–64.0	4.0	16.0
Ofloxacin	0.5–64.0	4.0	8.0
Levofloxacin	0.5–32.0	2.0	4.0
Sparfloxacin	0.125–16.0	1.0	2.0
Clinafloxacin	0.125–8.0	0.5	2.0
Ceftazidime	0.5–>256.0	64.0	256.0
Imipenem	16.0–256.0	256.0	256.0
Acinetobacter genospecies (52)[a]			
Trovafloxacin	0.016–32.0	0.06	16.0
Ciprofloxacin	0.03–>256.0	0.5	>256.0
Ofloxacin	0.016–64.0	0.5	32.0
Levofloxacin	0.016–32.0	0.5	32.0
Sparfloxacin	0.016–32.0	0.06	16.0
Clinafloxacin	0.016–8.0	0.125	8.0
Ceftazidime	0.25–>256.0	8.0	64.0
Imipenem	0.016–2.0	0.25	1.0
Alcaligenes faecalis-Alcaligenes odorans (27)			
Trovafloxacin	0.06–16.0	4.0	16.0
Ciprofloxacin	0.016–64.0	4.0	16.0
Ofloxacin	0.125–32.0	4.0	8.0
Levofloxacin	0.06–32.0	2.0	4.0
Sparfloxacin	0.03–16.0	2.0	8.0
Clinafloxacin	0.016–8.0	0.5	2.0
Ceftazidime	1.0–>256.0	8.0	256.0
Imipenem	0.25–256.0	1.0	8.0
Alcaligenes xylosoxidans (40)[b]			
Trovafloxacin	0.016–64.0	32.0	64.0
Ciprofloxacin	0.016–>256.0	8.0	32.0
Ofloxacin	2.0–128.0	16.0	32.0
Levofloxacin	1.0–64.0	8.0	32.0
Sparfloxacin	0.5–64.0	8.0	32.0
Clinafloxacin	0.25–16.0	2.0	8.0
Ceftazidime	2.0–>256.0	16.0	64.0
Imipenem	0.5–256.0	1.0	8.0
Moraxella-Oligella spp. (9)[c]			
Trovafloxacin	0.016–0.5	0.125	
Ciprofloxacin	0.016–2.0	0.06	
Ofloxacin	0.06–2.0	0.25	
Levofloxacin	0.06–1.0	0.06	
Sparfloxacin	0.016–0.25	0.06	
Clinafloxacin	0.016–0.25	0.016	
Ceftazidime	1.0–64.0	2.0	
Imipenem	0.06–8.0	0.125	
Pseudomonas stutzeri (10)			
Trovafloxacin	0.03–2.0	0.125	2.0
Ciprofloxacin	0.016–2.0	0.06	2.0
Ofloxacin	0.06–4.0	0.5	4.0
Levofloxacin	0.016–1.0	0.125	1.0
Sparfloxacin	0.016–0.5	0.125	0.5
Clinafloxacin	0.016–0.25	0.016	0.25
Ceftazidime	0.25–32.0	1.0	32.0
Imipenem	0.125–4.0	0.5	4.0
Brevundimonas diminuta (11)			
Trovafloxacin	1.0–8.0	4.0	8.0
Ciprofloxacin	8.0–32.0	16.0	32.0
Ofloxacin	8.0–64.0	16.0	32.0
Levofloxacin	2.0–32.0	8.0	16.0
Sparfloxacin	1.0–2.0	1.0	2.0
Clinafloxacin	0.5–4.0	2.0	4.0
Ceftazidime	64.0–>256.0	128.0	256.0
Imipenem	0.5–128.0	1.0	32.0
Flavobacterium odoratum (12)			
Trovafloxacin	0.03–4.0	0.25	4.0
Ciprofloxacin	0.125–128.0	4.0	64.0
Ofloxacin	0.5–128.0	4.0	64.0
Levofloxacin	0.5–64.0	2.0	32.0
Sparfloxacin	0.06–8.0	0.5	4.0
Clinafloxacin	0.06–8.0	1.0	4.0
Ceftazidime	2.0–>256.0	>256.0	>256.0
Imipenem	0.125–64.0	16.0	64.0
Chryseobacterium meningosepticum (10)			
Trovafloxacin	0.03–4.0	1.0	4.0
Ciprofloxacin	0.5–8.0	2.0	8.0
Ofloxacin	1.0–4.0	4.0	4.0
Levofloxacin	1.0–2.0	2.0	2.0
Sparfloxacin	0.016–4.0	0.5	4.0
Clinafloxacin	0.25–4.0	1.0	4.0
Ceftazidime	4.0–>256.0	128.0	>256.0
Imipenem	1.0–64.0	8.0	64.0
Chryseobacterium indologenes-Chryseobacterium gleum (9)			
Trovafloxacin	0.016–2.0	0.125	
Ciprofloxacin	0.25–8.0	1.0	
Ofloxacin	0.5–8.0	2.0	
Levofloxacin	0.25–4.0	1.0	
Sparfloxacin	0.016–2.0	0.25	
Clinafloxacin	0.125–2.0	0.5	
Ceftazidime	2.0–>256.0	16.0	
Imipenem	1.0–64.0	32.0	
Miscellaneous species (45)[d]			
Trovafloxacin	0.016–16.0	0.25	8.0
Ciprofloxacin	0.016–16.0	0.5	8.0
Ofloxacin	0.016–64.0	2.0	16.0
Levofloxacin	0.016–32.0	1.0	8.0
Sparfloxacin	0.016–64.0	0.25	2.0
Clinafloxacin	0.016–8.0	0.25	1.0
Ceftazidime	0.06–>256.0	16.0	128.0
Imipenem	0.016–256.0	0.5	32.0

Continued on following page

TABLE 1—*Continued*

Species and agent (no. of isolates tested)	MIC (μg/ml)		
	Range	50%	90%
All strains (458)			
Trovafloxacin	0.016–128.0	1.0	16.0
Ciprofloxacin	0.016–>256.0	2.0	16.0
Ofloxacin	0.016–256.0	2.0	32.0
Levofloxacin	0.016–128.0	1.0	16.0
Sparfloxacin	0.016–128.0	1.0	16.0
Clinafloxacin	0.016–32.0	0.5	4.0
Ceftazidime	0.06–>256.0	8.0	128.0
Imipenem	0.016–256.0	2.0	256.0

[a] A total of 38 *Acinetobacter baumannii* species and 14 non-*Acinetobacter baumannii* species.

[b] *Alcaligenes xylosoxidans* subsp. *denitrificans* ($n = 13$) and *Alcaligenes xylosoxidans* subsp. *xylosoxidans* ($n = 27$).

[c] *Moraxella osloensis* ($n = 6$), *Moraxella phenylpyruvica* ($n = 1$), *Moraxella nonliquefaciens* ($n = 1$), and *Oligella urethralis* ($n = 1$).

[d] *Shewanella putrefaciens* ($n = 6$), *Sphingomonas paucimobilis* ($n = 5$), *Pseudomonas alcaligenes* ($n = 3$), *Brevundimonas vesicularis* ($n = 4$), *Burkholderia pickettii* ($n = 2$), *Flavimonas oryzihabitans* ($n = 4$), *Chromobacterium violaceum* ($n = 2$), *Methylobacterium* spp. ($n = 2$), *Comamonas acidovorans* ($n = 3$), *Sphingobacterium multivorum* ($n = 6$), *Agrobacterium radiobacter* ($n = 1$), *Pseudomonas mendocina* ($n = 2$), *Ochrobactrum anthropi* ($n = 1$), *Pseudomonas pseudoalcaligenes* ($n = 1$), *Comamonas testosteroni* ($n = 1$), *Weeksella zoohelcum* ($n = 1$), and CDC group IVe ($n = 1$).

agar (BBL Microbiology Systems, Cockeysville, Md.). When testing *Moraxella* spp., 5% sheep blood was added to the medium. Suspensions with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by suspending growth from blood agar plates in 2 ml of Mueller-Hinton broth (BBL). Suspensions were further diluted 1:10 to obtain a final inoculum of 10^4 CFU/spot. Plates were inoculated with a Steers replicator and were incubated overnight in ambient air at 37°C. Standard quality control strains were included in each run. It is noteworthy that standardized methods have not been approved by NCCLS for most of the organisms tested in the current study. Although organisms do grow by the methods outlined by NCCLS, interpretive breakpoints have only been established for *P. aeruginosa* and, perhaps, *Acinetobacter* genospecies.

Broth MIC determination. For the five strains tested by the time-kill method, MICs were determined by the microdilution method recommended by NCCLS (27) by using cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.). Suspensions with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by suspending growth from blood agar plates in 2 ml of sterile saline. Suspensions were further diluted 1:10 to obtain a final inoculum of 5×10^5 CFU/well. Trays were incubated overnight in ambient air at 37°C. Standard quality control strains were included in each run.

Time-kill assays. For time-kill assays with the five strains mentioned above, glass tubes containing 5 ml of cation-adjusted Mueller-Hinton broth (Difco) with doubling antibiotic concentrations were inoculated with 5×10^5 to 5×10^6 CFU/ml and were incubated at 35°C in a shaking water bath. Antibiotic concentrations were chosen to comprise 3 doubling dilutions above and 4 doubling dilutions below the agar dilution MIC. The bacterial inoculum was prepared by diluting suspensions harvested from plates (the medium was as described above) in the same medium. The dilutions required to obtain the correct inoculum (5×10^5 to 5×10^6 CFU/ml) were determined by prior viability studies with each strain (29, 38).

To inoculate each tube of serially diluted antibiotic, 50 μl of diluted inoculum was delivered beneath the surface of the broth with a pipette. The tubes were then vortexed and their contents were plated for viability counts (0 h). Only tubes containing an initial inoculum within the range of 5×10^5 to 5×10^6 CFU/ml were acceptable.

Viability counts of antibiotic-containing suspensions were performed at 0, 6, 12, and 24 h by plating 0.1-ml aliquots of 10-fold dilutions from each tube in sterile Mueller-Hinton broth onto Trypticase soy agar–5% sheep blood agar plates (BBL). The plates used to recover organisms were incubated for up to 48 h. Colony counts were performed for plates yielding 30 to 300 colonies. The lower limit of sensitivity of colony counts was 300 CFU/ml (29, 38).

Time-kill assay results were analyzed by determining the number of strains which yielded a changes in the $\log_{10}$ CFU per milliliter of -1, -2, and -3 compared to the counts at time zero for all seven compounds at all five time periods. Antimicrobial agents were considered bactericidal at the lowest concentration that reduced the original inoculum by $>3 \log_{10}$ CFU/ml (99.9%) at each of the respective time periods and bacteriostatic if the inoculum was reduced by 0 to 3 $\log_{10}$ CFU/ml. With the inocula and viability count thresholds used in these studies, 99.9% killing, when present, could be readily detected. The problem of bacterial carryover was addressed as described previously (29, 38).

Checkerboard synergy testing. Checkerboard synergy testing was performed for 286 strains in microtiter trays with cation-supplemented Mueller-Hinton broth (Difco) (4, 5, 14). By using two microdilution trays, trovafloxacin was tested at 14 concentrations (0.004 to 32.0 μg/ml), ceftazidime and amikacin were each tested at 11 concentrations (0.06 to 64.0 μg/ml), and imipenem was tested at 11 concentrations (0.016 to 16.0 μg/ml). The trays were prepared with a 96-channel dispenser and were stored at −70°C until use. Trovafloxacin was dispensed alone in the first row and was combined with ceftazidime, amikacin, or imipenem in the remaining rows. Ceftazidime, amikacin, and imipenem were also dispensed alone in the first column. Inocula were prepared by suspending growth from blood agar plates in sterile saline to a density equivalent to that of a 0.5 McFarland standard and were diluted 1:10 to produce final inocula of 5×10^5 CFU/ml with a multipoint inoculator. Trays were incubated aerobically overnight. Standard quality control strains were included with each run. Fractional inhibitory concentrations (FICs) were calculated as the MIC of drug *A* or *B* in combination/the MIC of drug *A* or *B* alone, and the FIC index was obtained by adding the FIC values. FIC indices were interpreted as indicating synergism if the values were ≤ 0.5, additivity or indifference if the values were >0.5 to 4.0, and antagonism if the values were >4.0 (14).

Time-kill determinations. Twenty strains were tested by the time-kill method as described above. All four compounds were tested alone, and trovafloxacin was tested in combination with each of ceftazidime, amikacin, and imipenem. In each case, concentrations four times above and four times below the MICs were tested. Viability counts were performed at 0, 6, 12, and 24 h. Drug carryover was addressed as described previously (29, 38). Synergy was defined as a $\geq 2 \log_{10}$ decrease in viable count with the combination at 24 h compared to the viable count with the more active of the two compounds alone (4, 5, 9).

RESULTS

The results of agar dilution MIC testing of 458 strains are presented in Table 1. Different nonfermentative species differed in their susceptibilities to quinolone and nonquinolone agents. The overall respective MICs at which 50% of strains are inhibited (MIC_{50}s) and MIC_{90}s were as follows: trovafloxacin, 1.0 and 16.0 μg/ml; ciprofloxacin, 2.0 and 16.0 μg/ml; ofloxacin, 2.0 and 32.0 μg/ml; levofloxacin, 1.0 and 16.0 μg/ml; sparfloxacin, 1.0 and 16.0 μg/ml; clinafloxacin, 0.5 and 4.0 μg/ml; ceftazidime, 8.0 and 128.0 μg/ml; and imipenem, 2.0 and 256.0 μg/ml.

In general, clinafloxacin was the most active of all the quinolones tested. Trovafloxacin MIC_{90}s were ≤ 4.0 μg/ml for *P. aeruginosa*, *S. maltophilia*, *Flavobacterium odoratum*, and *Chryseobacterium meningosepticum*. For *Moraxella* spp., *Pseudomonas stutzeri*, and *Chryseobacterium indologenes-C. gleum*, trovafloxacin MICs were ≤ 2.0 μg/ml. A bimodal distribution of trovafloxacin MICs for *A. baumannii* species was observed,

TABLE 2. Broth dilution MICs for five strains tested by the time-kill method

Strain	MIC (μg/ml)							
	Trovafloxacin	Ciprofloxacin	Ofloxacin	Levofloxacin	Sparfloxacin	Clinafloxacin	Ceftazidime	Imipenem
P. aeruginosa	0.25	0.125	1.0	0.5	0.25	0.125	1.0	2.0
S. maltophilia	0.5	2.0	2.0	1.0	0.5	0.25	4.0	128.0
A. baumannii	0.03	0.5	0.5	0.5	0.03	0.125	32.0	0.5
B. cepacia	32.0	32.0	64.0	32.0	32.0	8.0	32.0	256.0
C. meningosepticum	4.0	4.0	2.0	2.0	4.0	1.0	4.0	1.0

TABLE 3. Microdilution MIC_{50}s and MIC_{90}s for 286 strains tested by the checkerboard titration method

Species	MIC_{50}/MIC_{90} (μg/ml)			
	Trova-floxacin	Ceftazi-dime	Amikacin	Imipenem
P. aeruginosa (60)[a]	0.5/1.0	4.0/>64.0	4.0/16.0	2.0/>4.0
P. fluorescens-P. putida (11)	0.5/8.0	32.0/>64.0	8.0/>16.0	4.0/>4.0
S. maltophilia (36)	2.0/4.0	64.0/>64.0	>16.0/>16.0	>4.0/>4.0
B. cepacia (32)	2.0/32.0	>64.0/>64.0	>16.0/>16.0	>4.0/>4.0
Acinetobacter spp. (35)	0.125/8.0	16.0/>64.0	4.0/>16.0	0.5/1.0
A. faecalis-A. odorans (23)	4.0/16.0	64.0/>64.0	>16.0/>16.0	1.0/4.0
A. xylosoxidans (24)	16.0/32.0	>64.0/>64.0	>16.0/>16.0	2.0/>4.0
Flavobacteria and chryseobacteria (22)[b]	0.25/4.0	>64.0/>64.0	>16.0/>16.0	4.0/>4.0
Moraxella (10)[c]	0.03/0.25	2.0/16.0	8.0/>16.0	0.06/0.5
Miscellaneous (33)[d]	0.25/4.0	16.0/>64.0	>16.0/>16.0	1.0/>4.0

[a] Values in parentheses are numbers of strains tested.
[b] *F. odoratum* (n = 10), *C. indologenes-C. gleum* (n = 6), and *C. meningosepticum* (n = 6).
[c] *Moraxella osloensis* (n = 8) and *Moraxella nonliquefaciens* (n = 2).
[d] *Sphingomonas paucimobilis* (n = 2), *Pseudomonas pseudoalcaligenes* (n = 5), *Pseudomonas stutzeri* (n = 5), *Pseudomonas mendocina* (n = 3), *Burkholderia pickettii* (n = 1), *Ochrobactrum anthropi* (n = 1), *Comamonas acidovorans* (n = 1), *Comamonas testosteroni* (n = 1), *Brevundimonas diminuta* (n = 3), *Brevundimonas vesicularis* (n = 3), *Sphingobacterium multivorum* (n = 4), *Flavimonas oryzihabitans* (n = 2), *Weeksella virosa* (n = 1), and CDC IV C-2 (n = 1).

with strains being either very susceptible (≤0.5 μg/ml) or very resistant (≥8.0 μg/ml). For all non-*A. baumannii* strains tested, trovafloxacin MICs were ≤0.5 μg/ml. Similar results were obtained with all quinolones tested. The MICs of ceftazidime (≤16.0 μg/ml) and imipenem (≤2.0 μg/ml) were also lower for non-*A. baumannii* strains than for *A. baumannii* strains. The other quinolones tested showed similar susceptibility patterns for the different nonfermentative groups, with the MICs of sparfloxacin and levofloxacin being lower than those of ciprofloxacin and ofloxacin. High ceftazidime MIC_{90}s (≥32.0 μg/ml) were observed for all nonfermentative species tested. Although for the majority of strains tested imipenem MICs were ≤8.0 μg/ml, high imipenem MICs were observed for many species, especially *S. maltophilia*, *B. cepacia*, *F. odoratum*, and *C. meningosepticum*. For *Alcaligenes xylosoxidans* strains, the MICs of all compounds were generally a few dilutions lower than those for *Alcaligenes faecalis-A. odorans* strains.

The broth dilution MICs for strains tested by the time-kill method are presented in Table 2. All strains except *B. cepacia* were inhibited by trovafloxacin and all quinolones (MICs, ≤4.0 μg/ml). Ceftazidime inhibited *P. aeruginosa*, *S. maltophilia*, and *C. meningosepticum* at ≤4.0 μg/ml, while imipenem was active against *P. aeruginosa*, *A. baumannii*, and *C. meningosepticum* at ≤2.0 μg/ml.

In time-kill studies, imipenem, trovafloxacin, and all quinolones except sparfloxacin at eight times the MIC yielded 99.9% killing for all five strains after 24 h; sparfloxacin yielded 99.9% killing for four of five strains after 24 h. Ceftazidime at eight times the MIC was bactericidal for only two of five strains after 24 h. Regrowth was observed with quinolones after 6 h for *P. aeruginosa* and *S. maltophilia.*

Microdilution test results for the 286 strains used in the checkerboard titrations are presented in Table 3. Trovafloxacin yielded the lowest MICs for fluorescent *Pseudomonas* group, *S. maltophilia*, *B. cepacia*, flavobacteria and chryseobacteria, *Moraxella* spp., and miscellaneous species, while imipenem yielded the lowest MICs for *Acinetobacter* and *Alcaligenes* strains.

Checkerboard titration test results for synergy are listed in Table 4. Trovafloxacin combined with ceftazidime, amikacin, and imipenem yielded FIC indices indicating synergism for 81 (28%), 41 (14%), and 40 (14%) strains, respectively, and FIC indices indicating additivity or indifference for 205 (72%), 245 (86%), and 246 (86%) strains, respectively. No FIC indices in the range indicating antagonism were observed. Trovafloxacin combined with ceftazidime yielded FIC indices indicating synergism for 32 of 26 *S. maltophilia* strains tested.

Time-kill test results for synergy are listed in Table 5. For most strains for which FIC indices indicated additivity or indifference by the checkerboard test, the FIC indices by the time-kill experiments indicated synergy. Synergy was particularly evident for *S. maltophilia* strains. In only one strain of *Brevundimonas vesicularis* (trovafloxacin-amikacin) did checkerboard titration yield synergy but time-kill study results were additive or indifferent.

DISCUSSION

Trovafloxacin is a broad-spectrum naphthyridone with activity against gram-positive aerobic cocci and rods, members of the family *Enterobacteriaceae*, and gram-positive and -negative anaerobes (10, 18, 37). In the current study, trovafloxacin MICs were low (generally ≤4.0 μg/ml) for *P. aeruginosa*, *S. maltophilia*, *Pseudomonas stutzeri*, *Moraxella* spp., and *Flavobacterium* and *Chryseobacterium* spp. In line with previous findings (34), *A. baumannii* strains were more resistant than non-*A. baumannii* strains to quinolone and nonquinolone agents. Fass and coworkers (16), in a study of 308 gram-negative nonfermenters, found trovafloxacin to be considerably more active than ciprofloxacin and ofloxacin against *S. maltophilia*, *A. baumannii*, and several less common species. However, trovafloxacin MICs for *P. aeruginosa* and *S. maltophilia* were higher than those reported here; strain differences may account for this discrepancy.

Checkerboard titrations revealed significant synergism between trovafloxacin and ceftazidime against *S. maltophilia*. No

TABLE 4. Results of checkerboard titration tests with 286 strains

Species	No. of strains for which FIC index was as indicated in tests with the following combinations[a]:								
	Trovafloxacin + ceftazidime			Trovafloxacin + amikacin			Trovafloxacin + imipenem		
	≤0.5	>0.5–4	>4	≤0.5	>0.5–4	>4	≤0.5	>0.5–4	>4
P. aeruginosa (60)[b]	17	43	0	5	55	0	14	46	0
P. fluorescens-P. putida (11)	5	6	0	3	8	0	4	7	0
S. maltophilia (36)	32	4	0	4	32	0	1	35	0
B. cepacia (32)	5	27	0	1	31	0	2	30	0
Acinetobacter spp. (35)	7	28	0	6	29	0	6	29	0
A. faecalis-A. odorans (23)	5	18	0	7	16	0	4	19	0
A. xylosoxidans (24)	1	23	0	3	21	0	1	23	0
Flavobacteria and chryseobacteria (22)	5	17	0	3	19	0	3	19	0
Moraxella spp. (10)	1	9	0	0	10	0	1	9	0
Miscellaneous (33)	3	30	0	9	24	0	4	29	0
All strains (286)	81	205	0	41	245	0	40	246	0

[a] An FIC index of ≤0.5 indicates synergism, an FIC index of >0.5 to 4.0 indicates additivity or indifference, and FIC index of >4.0 indicates antagonism.
[b] Values in parentheses are numbers of strains tested.

TABLE 5. Comparison of synergy testing by checkerboard titration and time-kill methods

Strain	MIC (μg/ml)				Trovafloxacin + ceftazidime		Trovafloxacin + amikacin		Trovaflxacin + imipenem	
	Trovafloxacin	Ceftazidime	Amikacin	Imipenem	C[a]	T MIC (μg/ml)[b]	C	T MIC (μg/ml)	C	T MIC (μg/ml)
P. aeruginosa	0.25	2	4	2	Ad	0.25/2	Ad	0.25/2	Ad	0.125/2
P. aeruginosa	0.125	16	4	4	Syn	0.03/4	Ad	0.03/1	Ad	0.06/0.5
P. aeruginosa	0.5	2	4	2	Ad	0.25/1	Ad	0.25/0.5	Ad	0.25/2
P. fluorescens-P. putida	0.125	>64	0.125	2	Ad	0.06/32	Ad	0.03/0.06	Ad	0.06/1
P. stutzeri	0.5	8	4	1	Ad	0.125/2	Syn	0.125/1	Ad	0.125/0.25
S. maltophilia	2	32	>16	>4	Syn	1/8	Ad	1/16	Ad	Ad
S. maltophilia	2	32	>16	>4	Syn	1/16	Ad	1/4	Ad	1/16
S. maltophilia	0.25	>64	>16	>4	Syn	0.125/64	Ad	Ad	Ad	Ad
B. cepacia	1	>64	4	4	Ad	0.25/32	Ad	0.25/1	Syn	0.25/0.5
B. cepacia	4	>64	32	4	Ad	0.5/2	Ad	0.5/8	Syn	0.5/1
B. cepacia	0.5	>64	4	4	Ad	0.125/32	Ad	0.25/1	Ad	0.25/2
A. baumannii	0.06	8	8	0.25	Ad	0.016/2	Ad	0.016/2	Ad	0.016/0.125
A. baumannii	1	64	>16	1	Ad	0.25/16	Ad	0.25/8	Syn	0.25/0.25
A. faecalis-A. odorans	2	>64	>16	>4	Ad	0.5/32	Ad	2/32	Ad	2/8
A. faecalis-A. odorans	4	>64	>16	4	Ad	1/16	Ad	2/16	Syn	2/2
A. xylosoxidans	8	>64	8	0.5	Ad	Ad	Ad	Ad	Ad	Ad
C. meningosepticum	8	>64	16	4	Ad	2/32	Ad	2/4	Syn	1/1
C. indologenes-C. gleum	0.5	64	>16	>4	Syn	0.25/32	Ad	0.25/16	Ad	0.25/4
S. multivorum	0.06	8	128	4	Ad	0.03/8.0	Ad	Ad	Ad	0.016/1
B. vesicularis	0.5	64	4	1	Ad	Ad	Syn	Ad	Ad	Ad

[a] C, Checkerboard titration method; Syn, synergism; Ad, additivity or indifference; A, antagonism.
[b] T, Time-kill method; values indicate the lowest concentration of each compound in the combination yielding sustained bactericidal activity (≥100 CFU/ml drop) at 24 h compared to the concentration of the more active drug; Ad, addition or indifference.

FIC indices indicating antagonism were observed for any of the combinations. Results of synergy testing revealed discrepant results between the checkerboard titration and time-kill experiments, with time-kill experiments yielding the most favorable results. This phenomenon has previously been observed for *Streptococcus pneumoniae* with β-lactams and glycopeptides (5), *Acinetobacter* genospecies with quinolones and amikacin (4), *P. aeruginosa* with β-lactams and an aminoglycoside (9), and members of the family *Enterobacteriaceae* with various drug combinations (9). The problem is further complicated by a lack of standardization of the two techniques to determine synergy (14).

Although synergy was found for all strains except *A. xylosoxidans* by the time-kill method, the results should be interpreted carefully, and the levels of the respective compounds achievable in human serum should also be considered. For example, a combination of trovafloxacin plus imipenem is not indicated for *S. maltophilia*, owing to inherent imipenem resistance in this species. Taken together, however, results of the checkerboard titration and time-kill method indicate clear synergy between trovafloxacin and ceftazidime for *S. maltophilia* strains. Given the tendency of *S. maltophilia* to develop resistance on exposure to ceftazidime, the clinical significance of the synergy observed with trovafloxacin is unknown. An animal model is being developed to investigate this phenomenon further.

Of the individual strains tested, the *S. maltophilia* strains tested revealed susceptibility and time-kill patterns typical of this strain: low quinolone MICs for the strains, especially those of trovafloxacin and sparfloxacin, higher ceftazidime MICs for the strains, and resistance to imipenem. Time-kill study results showed regrowth after 24 h. Bimodal susceptibility distributions were seen in *Acinetobacter* genospecies for all quinolones tested. Resistance in *B. cepacia* and *Alcaligenes* spp. has been described before, and the susceptibility patterns of *Flavobacterium* and *Chryseobacterium* spp. as well as those of less commonly occurring nonfermentative species are also in line with those in previous reports (1–4, 6–8, 11, 13, 15, 17, 19, 21, 22–26, 28, 30–36, 38–41, 43).

Among all quinolones tested, the MICs of clinafloxacin were the lowest for all strains, in agreement with previous studies (44). Of the other quinolones tested, sparfloxacin and levofloxacin had lower MICs, similar to those of trovafloxacin, for a bacterial population substantially different from that studied previously in our laboratory (38).

Widespread resistance to ceftazidime, together with significantly increased bactericidal compared to bacteriostatic values for most strains tested, limits the use of this compound for the treatment of infections caused by nonfermenters. In the current study, ceftazidime MICs were ≥32.0 μg/ml for 17 of 89 (19.1%) *P. aeruginosa* strains. Widespread imipenem resistance in species other than those with known inherent resistance (*S. maltophilia*, flavobacteria, and chryseobacteria) mandates susceptibility testing with this compound in all cases of serious infections caused by gram-negative nonfermenters. Different susceptibilities in different species mandates susceptibility testing of all clinically significant gram-negative nonfermenters. The problem is complicated by the fact that no approved breakpoints are available for nonfermenters other than *P. aeruginosa* and perhaps *Acinetobacter*.

In two previous studies, synergy between each of three quinolones (levofloxacin, ofloxacin, and ciprofloxacin) and amikacin has been demonstrated for *Acinetobacter* genospecies for which quinolone MICs were ≤2.0 μg/ml by time-kill (but not checkerboard) testing (4, 12). The current study indicates a possible place for combination therapy with trovafloxacin and other agents for selected nonfermentative strains and suggests that other quinolones may be combined with amikacin, ceftazidime, or imipenem in selected cases. The latter hypothesis requires laboratory testing for confirmation.

Clinical studies are required to test the relevance of the increased activities of trovafloxacin (with and without other

agents), sparfloxacin, clinafloxacin, and levofloxacin against gram-negative nonfermenters. However, these will be difficult to achieve, given the infrequency with which many of these organisms can be definitively implicated in human infection versus colonization. Animal models may help in this regard and need to be developed. This study has highlighted the widespread resistance of all nonfermentative strains tested to quinolones and nonquinolones and demonstrates the need for the development of agents with activities against this group of organisms.

ACKNOWLEDGMENT

This study was supported by grants from Pfizer, Inc., New York, N.Y.

REFERENCES

1. **Appelbaum, P. C., S. K. Spangler, and L. Sollenberger.** 1986. Susceptibility of nonfermentative gram-negative bacteria to ciprofloxacin, norfloxacin, amifloxacin, pefloxacin and cefpirome. J. Antimicrob. Chemother. **18:**675–679.
2. **Appelbaum, P. C., S. K. Spangler, and T. Tamarree.** 1988. Susceptibility of 310 nonfermentative gram-negative bacteria to aztreonam, carumonam, ciprofloxacin, ofloxacin and fleroxacin. Chemotherapy (Basel) **34:**40–45.
3. **Appelbaum, P. C., J. Tamim, J. Stavitz, R. C. Aber, and G. A. Pankuch.** 1982. Sensitivity of 341 non-fermentative gram-negative bacteria to seven beta-lactam antibiotics. Eur. J. Clin. Microbiol. **1:**159–165.
4. **Bajaksouzian, S., M. A. Visalli, M. R. Jacobs, and P. C. Appelbaum.** 1997. Activities of levofloxacin, ofloxacin, and ciprofloxacin, alone and in combination with amikacin, against acinetobacters as determined by checkerboard and time-kill studies. Antimicrob. Agents Chemother. **41:**1073–1076.
5. **Bajaksouzian, S., M. A. Visalli, M. R. Jacobs, and P. C. Appelbaum.** 1996. Antipneumococcal activities of cefpirome and cefotaxime, alone and in combination with vancomycin and teicoplanin, determined by checkerboard and time-kill methods. Antimicrob. Agents Chemother. **40:**1973–1976.
6. **Bergogne-Berezin, E., and M. L. Joly-Guillou.** 1986. Comparative activity of imipenem, ceftazidime and cefotaxime against *Acinetobacter calcoaceticus*. J. Antimicrob. Chemother. **18**(Suppl. E):35–39.
7. **Bizet, C., E. Tekaia, and A. Philippon.** 1993. In-vitro susceptibility of *Alcaligenes faecalis* compared with those of other *Alcaligenes* spp. to antimicrobial agents including seven β-lactams. J. Antimicrob. Chemother. **32:**907–910.
8. **Cantón, E., J. Pemán, M. T. Jimenez, M. S. Ramón, and M. Gobernado.** 1992. In vitro activity of sparfloxacin compared with those of five other quinolones. Antimicrob. Agents Chemother. **36:**558–565.
9. **Cappelletty, D. M., and M. J. Rybak.** 1996. Comparison of methodologies for synergism testing of drug combinations against resistant strains of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **40:**677–683.
10. **Child, J., J. Andrews, F. Boswell, N. Brenwald, and R. Wise.** 1995. The in-vitro activity of CP 99,219, a new naphthyridone antimicrobial agent: a comparison with fluoroquinolone agents. J. Antimicrob. Chemother. **35:**869–876.
11. **Decré, D., G. Arlet, C. Danglot, J.-C. Lucet, G. Fournier, E. Bergogne-Bérézin, and A. Philippon.** 1992. A β-lactamase-overproducing strain of *Alcaligenes denitrificans* subsp. *xylosoxidans* isolated from a case of meningitis. J. Antimicrob. Chemother. **30:**769–779.
12. **Decré, D., C. Benoit, M. L. Joly-Guillou, E. Bergogne-Berezin, and A. Bryskier.** 1995. In vitro activity and bactericidal activity of levofloxacin (levx) alone or in combination with amikacin against *Acinetobacter* spp in comparison with ofloxacin (ofl) and ciprofloxacin (cip), abstr. E100, p. 103. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
13. **Dholakia, N., K. V. I. Rolston, D. H. Ho, B. LeBlanc, and G. P. Bodey.** 1994. Susceptibilities of bacterial isolates from patients with cancer to levofloxacin and other quinolones. Antimicrob. Agents Chemother. **38:**848–852.
14. **Eliopoulos, G. M., and R. C. Moellering, Jr.** 1996. Antimicrobial combinations, p. 330–396. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore, Md.
15. **Fass, R. J., and J. Barnishan.** 1980. In vitro susceptibilities of nonfermentative gram-negative bacilli other than *Pseudomonas aeruginosa* to 32 antimicrobial agents. Rev. Infect. Dis. **2:**841–853.
16. **Fass, R. J., J. Barnishan, M. C. Solomon, and L. W. Ayers.** 1996. In vitro activities of quinolones, β-lactams, tobramycin, and trimethoprim-sulfamethoxazole against nonfermentative gram-negative bacteria. Antimicrob. Agents Chemother. **40:**1412–1418.
17. **Fu, K. P., S. C. Lafredo, B. Foleno, D. M. Isaacson, J. F. Barrett, A. J. Tobia, and M. E. Rosenthale.** 1992. In vitro and in vivo antibacterial activities of levofloxacin (*l*-ofloxacin), an optically active ofloxacin. Antimicrob. Agents Chemother. **36:**860–866.
18. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509–519. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
19. **Glupczynski, Y., W. Hensen, J. Freney, and E. Yourrasowsky.** 1988. In vitro susceptibility of *Alcaligenes denitrificans* subsp. *xylosoxidans* to 24 antimicrobial agents. Antimicrob. Agents Chemother. **32:**276–278.
20. **Gooding, B. G., and R. N. Jones.** 1993. In vitro antimicrobial activity of CP-99,219, a novel azabicyclo-naphthyridone. Antimicrob. Agents Chemother. **37:**349–353.
21. **Husson, M. O., D. Izard, L. Bouillet, and H. Leclerc.** 1985. Comparative *in-vitro* activity of ciprofloxacin against non-fermenters. J. Antimicrob. Chemother. **15:**457–462.
22. **Joly-Guillou, M. L., and E. Bergogne-Bérézin.** 1992. In-vitro activity of sparfloxacin, pefloxacin, ciprofloxacin and temafloxacin against clinical isolates of *Acinetobacter* spp. J. Antimicrob. Chemother. **29:**466–468.
23. **Khardori, N., A. Reuben, B. Rosenbaum, K. Rolston, and G. P. Bodey.** 1990. In vitro susceptibility of *Xanthomonas* (*Pseudomonas*) *maltophilia* to newer antimicrobial agents. Antimicrob. Agents Chemother. **34:**1609–1610.
24. **Lecso-Bornet, M., J. Pierre, D. Sarkis-Karam, S. Lubera, and E. Bergogne-Berezin.** 1992. Susceptibility of *Xanthomonas maltophilia* to six quinolones and study of outer membrane proteins in resistant mutants selected in vitro. Antimicrob. Agents Chemother. **36:**669–671.
25. **Louie, A., A. L. Baltch, W. J. Ritz, and R. P. Smith.** 1991. Comparative in vitro susceptibilities of *Pseudomonas aeruginosa*, *Xanthomonas maltophilia*, and *Pseudomonas* spp. to sparfloxacin (CI-978, AT-4140, PD 131501) and reference antimicrobial agents. J. Antimicrob. Chemother. **27:**793–799.
26. **Mensah, K., A. Philippon, C. Richard, and P. Névot.** 1990. Susceptibility of *Alcaligenes denitrificans* subspecies *xylosoxidans* to beta-lactam antibiotics. Eur. J. Clin. Microbiol. Infect. Dis. **9:**405–409.
27. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS publication no. M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
28. **Neu, H. C., and N.-X. Chin.** 1989. In vitro activity of *S*-ofloxacin. Antimicrob. Agents Chemother. **33:**1105–1107.
29. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1994. Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin, and vancomycin by using time-kill methodology. Antimicrob. Agents Chemother. **38:**2065–2072.
30. **Raimondi, A., F. Moosdeen, and J. D. Williams.** 1986. Antibiotic resistance pattern of *Flavobacterium meningosepticum*. Eur. J. Clin. Microbiol. Infect. Dis. **5:**461–463.
31. **Rolston, K. V. I., and G. P. Bodey.** 1986. In vitro susceptibility of *Acinetobacter* species to various antimicrobial agents. Antimicrob. Agents Chemother. **30:**769–770.
32. **Rolston, K. V. I., D. H. Ho, B. LeBlanc, and G. P. Bodey.** 1993. In vitro activities of antimicrobial agents against clinical isolates of *Flavimonas oryzihabitans* obtained from patients with cancer. Antimicrob. Agents Chemother. **37:**2504–2505.
33. **Rolston, K. V. I., H. Nguyen, M. Messer, B. LeBlanc, D. H. Ho, and G. P. Bodey.** 1990. In vitro activity of sparfloxacin (CI-978; AT-4140) against clinical isolates from cancer patients. Antimicrob. Agents Chemother. **34:**2263–2266.
34. **Seifert, H., R. Baginski, A. Schulze, and G. Pulverer.** 1993. Antimicrobial susceptibility of *Acinetobacter* species. Antimicrob. Agents Chemother. **37:**750–753.
35. **Simor, A. E., L. Louie, and M. Louie.** 1994. In vitro susceptibility of *Acinetobacter baumannii* to biapenem, piperacillin/tazobactam and thirteen other antimicrobial agents. Eur. J. Clin. Microbiol. Infect. Dis. **13:**521–523.
36. **Smalley, D. L., V. R. Hansen, and V. S. Baselski.** 1983. Susceptibility of *Pseudomonas paucimobilis* to 24 antimicrobial agents. Antimicrob. Agents Chemother. **23:**161–162.
37. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** 1994. Activity of CP 99,219 compared with those of ciprofloxacin, grepafloxacin, metronidazole, cefoxitin, piperacillin and piperacillin/tazobactam against 489 anaerobes. Antimicrob. Agents Chemother. **38:**2471–2476.
38. **Spangler, S. K., M. A. Visalli, M. R. Jacobs, and P. C. Appelbaum.** 1996. Susceptibilities of non-*Pseudomonas aeruginosa* gram-negative nonfermentative rods to ciprofloxacin, ofloxacin, levofloxacin, D-ofloxacin, sparfloxacin, ceftazidime, piperacillin, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, and imipenem. Antimicrob. Agents Chemother. **40:**772–775.
39. **Tanaka, M., M. Otsuki, T. Une, and T. Nishino.** 1990. In-vitro and in-vivo activity of DR-3355, an optically active isomer of ofloxacin. J. Antimicrob. Chemother. **26:**659–666.
40. **Vartivarian, S., E. Anaissie, G. Bodey, H. Sprigg, and K. Rolston.** 1994. A changing pattern of susceptibility of *Xanthomonas maltophilia* to antimicrobial agents: implications for therapy. Antimicrob. Agents Chemother. **38:**624–627.
41. **Visser, M. R., M. Rozenberg-Arska, H. Beumer, I. M. Hoepelman, and J. Verhoef.** 1991. Comparative in vitro antibacterial activity of sparfloxacin

(AT-4140; RP 64206), a new quinolone. Antimicrob. Agents Chemother. **35:**858–868.

42. **von Graevenitz, A.** 1995. *Acinetobacter*, *Alcaligenes*, *Moraxella*, and other nonfermentative gram-negative bacteria, p. 520–532. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
43. **von Graevenitz, A., and C. Bucher.** 1982. The effect of *N*-formimidoyl thienamycin, ceftazidime, cefotiam, ceftriaxone and cefotaxime on non-fermentative gram-negative rods, Aeromonas, Plesiomonas and Enterobacter agglomerans. Infection **10:**293–298.
44. **Wise, R., J. P. Ashby, and J. M. Andrews.** 1988. In vitro activity of PD 127,391, an enhanced-spectrum quinolone. Antimicrob. Agents Chemother. **32:**1251–1256.