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In Vitro Activities of Newer Quinolones against *Bacteroides* Group Organisms

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The activities of BMS-284576, clinafloxacin, moxifloxacin, sitafloxacin, trovafloxacin, imipenem, cefoxitin, and clindamycin against 589 *Bacteroides fragilis* group isolates were determined. The activity of BMS-284576 was comparable to that of trovafloxacin. Sitafloxacin and clinafloxacin were the most active quinolones, and moxifloxacin was the least active. *B. fragilis* was the most susceptible of the species, and *Bacteroides vulgatus* was the most resistant. Association of specific antibiotic resistance with *Bacteroides* species was noted for all quinolones.

The increasing resistance of the *Bacteroides fragilis* group isolates to β-lactam antibiotics and other agents that act against anaerobic bacteria has established a need for newer antibiotics effective in the treatment of anaerobic infections (1, 2, 13, 15). The use of quinolones as monotherapy for mixed infections has been limited by their lack of activity against anaerobic pathogens (5, 7, 8). The major quinolones in clinical use today, such as ciprofloxacin, ofloxacin, levofloxacin, and sparfloxacin, have limited in vitro activity against anaerobic bacteria (3, 4, 5, 8). Trovafloxacin is the only quinolone approved for use against anaerobic bacteria; however due to toxicity, the use of this agent has been limited (11, 14). There are several newer quinolones in clinical development that exhibit activity against anaerobic bacteria, in particular the *B. fragilis* group (3, 6, 10, 17). These newer quinolones with activity against a broad range of aerobic and anaerobic bacteria could be ideal agents for potential use as monotherapy against mixed infections, such as intra-abdominal sepsis.

This study compares the in vitro activities of five quinolones, BMS-284756, clinafloxacin, moxifloxacin, sitafloxacin, and trovafloxacin, and emphasizes the association of specific drug resistance to particular species within the *B. fragilis* group (12, 13). Three antibiotics with known and differing activities against anaerobic bacteria, imipenem, cefoxitin, and clindamycin, were included for comparison in the evaluations (12, 13, 15).

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Five hundred and eighty-nine isolates were selected from a collection of strains referred from 1999 to 2000 by 10 medical centers from diverse geographical areas in the United States participating in a multicenter survey on the susceptibility of the *B. fragilis* group. The identification of the isolates was confirmed using API Anident and/or routine methodology when applicable (9, 16).

Standard powders of the antibiotics were provided by their manufacturers as follows: BMS-284756, Bristol-Myers Squibb, Princeton, N.J; clinafloxacin, Parke-Davis, Morris Plains, N.J.; moxifloxacin, Bayer Corporation, West Haven, Conn.; sitafloxacin, Daiichi Pharmaceuticals, Montvale, N.J.; trovafloxacin, Pfizer, Inc., New York, N.Y.; cefoxitin and imipenem, Merck and Company, Rahway, N.J.; and clindamycin, Pharmacia UpJohn, Kalamazoo, Mich. Stock solutions of the antibiotics were prepared at 10 times the testing concentration and kept frozen at −70°C until the day of the test. The range of concentrations at which the antibiotics were tested is shown in Table 1.

The MICs of the antibiotics were determined by the agar dilution method following National Committee for Clinical Laboratory Standards (NCCLS) recommendations (11). The medium used was brucella blood agar supplemented with 5 μg of hemin and 1 μg of vitamin K1 per ml and 5% (vol/vol) lysed sheep blood. The antibiotic-containing plates were prepared in-house on the day of the test by adding twofold serial dilutions of the corresponding antibiotic to molten agar. The bacteria were grown to logarithmic phase in brain heart infusion supplemented broth (BHIS), and their turbidity was adjusted to that of a 0.5 McFarland standard (~10^8 CFU/ml). A Steers replicator was used to deliver the inocula (10^5 CFU/spot) onto the surface of the agar plates. After the inocula had dried, the plates were inverted and incubated for 42 to 48 h at 37°C in an

TABLE 1. Susceptibilities of 589 *B. fragilis* group isolates by species

Species (no. of isolates) and antibiotic	MIC range	Geometric mean MIC	MIC_{50}	MIC_{90}	% of isolates inhibited at breakpoint[a] of:			
					≥1 μg/ml	≥2 μg/ml	≥4 μg/ml	≥8 μg/ml
B. fragilis group (589)								
BMS-284756	0.06–64	1.3	1	8	62.8	43.0	22.8	11.5
Clinafloxacin	0.06–32	0.7	0.5	4	41.9	34.1	14.8	6.1
Moxifloxacin	0.06–128	2.7	2	32	78.4	61.6	41.5	31.3
Sitafloxacin	0.06–64	0.5	0.5	2	34.6	21.9	9.5	5.6
Trovafloxacin	0.06–32	1.7	1	8	69.6	46.9	33.4	24.3
Imipenem	0.125–16	0.4	0.5	1				0.2
Cefoxitin	2–256	17.8	16	64				10.0
Clindamycin	0.5–256	3.7	1	256				26.1
B. distasonis (36)								
BMS-284756	0.06–32	1.8	2	8	80.6	52.8	36.1	16.7
Clinafloxacin	0.125–16	0.9	0.5–1	8	50.0	36.1	27.8	11.1
Moxifloxacin	0.125–64	2.8	1–2	32	83.3	50.0	38.9	33.3
Sitafloxacin	0.06–16	0.6	0.25–0.5	4	36.1	33.3	13.9	8.3
Trovafloxacin	0.06–16	2.6	2	16	86.1	72.2	44.4	33.3
Imipenem	0.25–16	1.0	1	2				2.8
Cefoxitin	2–256	27.4	32	64				25.0
Clindamycin	0.5–256	9.7	4	256				38.9
B. fragilis (288)								
BMS-284756	0.06–32	0.9	0.5	4	49.0	34.7	13.9	4.9
Clinafloxacin	0.06–16	0.5	0.25	2	37.2	28.8	7.6	2.8
Moxifloxacin	0/06–64	1.5	1	8	61.0	40.8	32.1	25.1
Sitafloxacin	0.06–16	0.3	0.25	1	21.5	6.9	2.4	1.4
Trovafloxacin	0.25–16	1.3	0.5	8	49.3	36.8	29.9	20.1
Imipenem	0.125–8	0.4	0.25	1				0
Cefoxitin	2–128	14.4	16	32				3.5
Clindamycin	0.5–256	1.8	0.5	256				16.3
B. ovatus (61)								
BMS-284756	0.25–32	1.7	1	8	75.4	49.2	24.6	14.8
Clinafloxacin	0.125–32	0.8	0.5	4	42.6	31.1	14.8	3.3
Moxifloxacin	0.5–64	4.1	4	32	95.1	91.8	55.7	27.9
Sitafloxacin	0.06–32	0.8	0.5	4	42.6	27.9	13.1	4.9
Trovafloxacin	0.5–16	1.9	2	8	93.4	60.7	23.0	14.8
Imipenem	0.125–4	0.5	0.5	1				0
Cefoxitin	4–128	24.1	32	64				14.8
Clindamycin	0.5–256	12.5	4	256				44.3
B. thetaiotaomicron (136)								
BMS-284756	0.25–64	1.6	1	8	72.1	44.1	25.0	14.7
Clinafloxacin	0.125–32	0.8	0.5	4	40.4	36.0	16.2	6.6
Moxifloxacin	0.5–64	4.2	2	32	97.1	84.6	44.9	35.3
Sitafloxacin	0.125–16	0.8	0.5	4	44.9	33.1	15.4	8.8
Trovafloxacin	0.5–16	2.0	1	8	89.7	46.3	33.8	23.5
Imipenem	0.125–4	0.5	0.5	1				0
Cefoxitin	2–128	25.4	32	64				18.4
Clindamycin	0.5–256	7.5	4	256				33.1
B. uniformis (11)								
BMS-284756	0.5–8	2.1	2	4	90.9	72.7	36.4	9.1
Clinafloxacin	0.5–4	1.4	2	4	72.7	54.5	18.2	0
Moxifloxacin	1–32	6.2	8	32	100	90.9	72.7	54.5
Sitafloxacin	0.25–8	0.9	1	4	54.5	27.3	18.2	9.1
Trovafloxacin	1–8	4.0	8	8	100	81.8	63.9	54.5
Imipenem	0.25–1	0.3	0.25	0.5				0
Cefoxitin	2–32	10.3	16	32				0
Clindamycin	0.5–256	5.2	2	256				36.4
B. vulgatus (35)								
BMS-284756	0.25–64	4.2	4	32	85.7	74.3	62.9	37.1
Clinafloxacin	0.125–32	2.2	2	16	71.4	68.6	45.7	31.4
Moxifloxacin	1–128	15.1	32	128	100	88.6	74.3	65.7
Sitafloxacin	0.25–64	2.2	2	16	74.3	68.6	31.4	25.7
Trovafloxacin	0.5–32	4.3	8	16	80	74.3	67.7	62.9
Imipenem	0.125–4	0.6	0.5	2				0
Cefoxitin	4–128	10.2	8	32				8.6
Clindamycin	0.5–256	5.5	0.5	256				37.1

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TABLE 1—*Continued*

Species (no. of isolates) and antibiotic	MIC range	Geometric mean MIC	MIC_{50}	MIC_{90}	% of isolates inhibited at breakpoint[a] of:			
					≥1 μg/ml	≥2 μg/ml	≥4 μg/ml	≥8 μg/ml
Other *Bacteroides* species[b] (22)								
BMS-284756	0.5–32	1.8	1	16	72.7	45.5	27.3	22.7
Clinafloxacin	0.125–8	0.7	0.5	4	36.4	31.8	27.3	9.1
Moxifloxacin	0.5–64	3.0	2	16	90.9	68.2	40.9	27.3
Sitafloxacin	0.125–16	0.8	0.5	2	45.5	36.4	9.1	4.5
Trovafloxacin	0.5–16	1.7	1	8	86.4	40.9	22.7	18.2
Imipenem	0.125–4	0.4	0.5	1				0
Cefoxitin	4–256	20.6	16–32	64				13.6
Clindamycin	0.5–256	2.0	0.5	256				18.2

[a] NCCLS-established guidelines for breakpoints for resistance for anaerobic bacteria follow: trovafloxacin, 8 μg/ml; clindamycin, 4 mg/ml; imipenem, 16 mg/ml; and cefoxitin, 64 mg/ml. NCCLS has not established breakpoint guidelines for BMS-284756, clinafloxacin, moxifloxacin, and sitafloxacin.
[b] Includes *B. caccae, B. eggerthii, B. merdae,* and *B. stercoralis.*

anaerobic chamber. The MIC endpoint was read at the concentration where a marked reduction occurs in the appearance of growth on the test plate compared to that of growth on the anaerobic control plate. *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were used as controls in all the test runs.

The susceptibilities of the isolates, listed by species, are shown in Table 1. The results are expressed as the geometric mean MIC, the MICs at which 50 and 90% of the strains were inhibited (MIC_{50} and MIC_{90}, respectively), and the percentage of isolates resistant at the specified breakpoint. The percentages of resistance for BMS-284756, clinafloxacin, moxifloxacin, and sitafloxacin were compared at breakpoints of 1, 2, 4, and 8 μg/ml. For purpose of comparison, the four breakpoints were also used for trovafloxacin, although the NCCLS recommendation for resistance breakpoint for this agent is 8 μg/ml. Percent resistance for the three reference agents, imipenem, cefoxitin, and clindamycin, was calculated at the recommended NCCLS breakpoint for anaerobic bacteria.

Against all the species of the *B. fragilis* group, sitafloxacin showed the greatest activity among the five quinolones evaluated. The geometric mean MIC and MIC_{90} of sitafloxacin were 0.5 and 2 μg/ml, respectively. Clinafloxacin ranked a very close second with a geometric mean MIC and MIC_{90} of 0.7 and 4 μg/ml, respectively. Against this group of isolates, BMS-284756 and trovafloxacin showed very similar activities; the MIC_{90} for both agents was 8 μg/ml, and the geometric mean MICs were 1.3 and 1.7 μg/ml, respectively. Against the *B. fragilis* group isolates as well as against all the isolates from individual species, moxifloxacin was the least active of the quinolones showing resistance rates (at a breakpoint of 4 μg/ml) greater than 30%. The three reference agents, clindamycin, imipenem, and cefoxitin, exhibited resistance rates against the isolates of the *B. fragilis* group comparable to published information: 24, 0.2, and 10%, respectively (12, 13, 15).

Analysis of the data by species showed that the quinolones were most active against *B. fragilis*; the lowest ranges of geometric mean MICs and MIC_{90}s (0.3 to 1.5 μg/ml and 1 to 8 μg/ml, respectively) were noted. By contrast, the quinolones were least active against *B. vulgatus* isolates (geometric mean MICs ranged from 2.2 to 15.1 μg/ml, and MIC_{90}s ranged from 16 to 128 μg/ml). Against *B. vulgatus*, using a breakpoint of 4 μg/ml (8 μg/ml for trovafloxacin), the resistance rates of the quinolones were considerably higher than those of clindamycin (the least active of the reference agents) with the exception of sitafloxacin. In addition to resistance to *B. vulgatus* and using the same breakpoint of 4 μg/ml, resistance rates greater than 10% were observed for sitafloxacin and clinafloxacin against *Bacteroides distasonis*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, and *Bacteroides uniformis,* while the resistance rates for BMS-284576 and trovafloxacin against these same species were greater than 20%. Although only a small number of other, less commonly isolated *Bacteroides* species (including *B. caccae*, *B. eggerthii*, *B. merdae*, and *B. sterocoralis*) were tested, resistance rates for all the quinolones, with the exception of sitafloxacin, were also higher than 20%.

On the basis of their in vitro activity, some of these newer quinolones could be good alternatives to agents included in the armamentarium against anaerobic bacteria. However, we emphasize the importance of correct species identification of the isolates if empirical data are to be used, as there is a marked relationship between a particular species and the in vitro activity of the drugs. Caution should also be used because of the emergence of resistance within the *B. fragilis* group isolates to this class of antibiotics, as noted in previous studies (12, 13).

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