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Besifloxacin, a Novel Fluoroquinolone, Has Broad-Spectrum In Vitro Activity against Aerobic and Anaerobic Bacteria▽

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The antibacterial spectrum of besifloxacin, a novel fluoroquinolone recently approved for treatment of ocular infections, was studied using 2,690 clinical isolates representing 40 species. Overall, besifloxacin was the most potent agent tested against gram-positive pathogens and anaerobes and was generally equivalent to comparator fluoroquinolones in activity against most gram-negative pathogens. Besifloxacin demonstrated potent, broad-spectrum activity, which was particularly notable against gram-positive and gram-negative isolates that were resistant to other fluoroquinolones and classes of antibacterial agents.

Bacterial conjunctivitis is an inflammation of the transparent mucous membrane covering the globe of the eye. This common ocular surface infection is caused by a broad variety of bacteria and is usually treated with broad-spectrum topical ophthalmic antibacterials (1, 13, 19, 20). The increasing prevalence of drug-resistant ocular isolates highlights the need for the development of new agents for the treatment of bacterial infections (8). Besifloxacin (Fig. 1) is a novel 8-chloro-fluoroquinolone agent with potent, bactericidal activity against prevalent and drug-resistant pathogens (6). Clinical development of the agent for use as a topical ophthalmic agent has been completed, and the agent has demonstrated consistent safety and efficacy in three bacterial conjunctivitis clinical trials (14, 16, 21). Pharmacokinetic studies demonstrated that after a single topical dose of 0.6% besifloxacin ophthalmic suspension, mean besifloxacin levels in human tears ranged from 610 μg/ml at 10 min postadministration to 1.6 μg/ml at 24 h postadministration (12). Here, the in vitro activity of besifloxacin against a broad range of aerobic and anaerobic bacterial species, including problematic drug-resistant strains, was evaluated.

Totals of 2,535 aerobic and 155 anaerobic bacterial clinical isolates from Eurofins Medinet (Chantilly, VA) were selected for the study. The majority of isolates were from 2005 to 2008, and wherever possible, isolates were from ocular and respiratory specimens of U.S. origin. Susceptibility testing was conducted per Clinical and Laboratory Standards Institute reference methods (9–11).

Activity of besifloxacin against gram-positive aerobes. Against *Enterococcus faecalis* and *E. faecium*, including vancomycin-resistant enterococci, besifloxacin was more potent than the comparator fluoroquinolones, as well as azithromycin, vancomycin, and tobramycin (Table 1). Besifloxacin further demonstrated excellent activity against *Listeria monocytogenes*, similar to that of tobramycin and penicillin and better than that observed with comparator fluoroquinolones.

For *Staphylococcus aureus* and *S. epidermidis*, previously reported MIC_{50}s/MIC_{90}s were consistent with current besifloxacin values for quinolone-susceptible and quinolone-resistant subsets (3, 5, 14). Besifloxacin was especially potent against ciprofloxacin-resistant isolates; for example, the MIC_{90}s for non-ciprofloxacin-susceptible *S. aureus* were 4 μg/ml for besifloxacin but out of range (>8 μg/ml) for all other fluoroquinolones. A similar trend was noted for *S. epidermidis*. As indicated in Table 1, 21.1% to 83.3% of the *S. aureus* and *S. epidermidis* ocular isolates tested were also methicillin (meticillin) resistant; however, resistance to methicillin did not affect the susceptibility to besifloxacin (data not shown). Against five other staphylococcal species, besifloxacin was generally the most active fluoroquinolone tested, even in instances where the test group contained a significant fraction of isolates that were not susceptible to the comparator agents. For staphylococci overall, besifloxacin was the most active agent tested.

Besifloxacin also demonstrated potent activity against a broad range of streptococci, including *Streptococcus agalactiae*, Lancefield group C, F, and G streptococci, *S. pneumoniae*, *S. pyogenes*, and viridans streptococci. For *S. pneumoniae*, besifloxacin MIC_{90}s were 0.06 μg/ml for levofloxacin-susceptible isolates and 0.5 μg/ml for non-levofloxacin-susceptible isolates (Table 1). Comparator fluoroquinolones had MIC_{90}s that were two- and eightfold higher, respectively. These results were similar to those from previous studies that reported overall *S. pneumoniae* besifloxacin MIC_{90}s of ≤0.12 μg/ml and showed that penicillin-resistant strains were equally susceptible to besifloxacin (5, 15). For all other streptococci tested, which in-

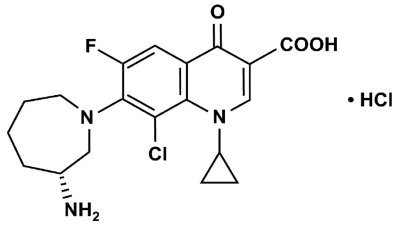

FIG. 1. Chemical structure of besifloxacin hydrochloride.

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TABLE 1. Activities of besifloxacin and comparators against gram-positive bacteria

Species or bacterial group (no. of isolates and phenotype[a]) and test drug	MIC (μg/ml)[b]			
	Range	50%	90%	%S[c]
Enterococcus faecalis (*n* = 130; 20.0% VRE)				
Besifloxacin	≤0.03–8	0.12	2	NA
Moxifloxacin	≤0.03–32	0.25	16	NA
Ciprofloxacin	0.12–>128	1	64	58.5
Levofloxacin	0.12–128	1	64	58.5
Azithromycin	0.25–>128	>128	>128	NA
Tobramycin	2–>128	16	>128	NA
Vancomycin	≤0.5–>32	1	>32	80.0
Enterococcus faecium (*n* = 110; 12.7% VRE)				
Besifloxacin	0.06–32	2	8	NA
Moxifloxacin	0.12–64	16	64	NA
Ciprofloxacin	0.25–>128	64	>128	18.2
Levofloxacin	0.25–>128	32	128	30.9
Azithromycin	0.06–>128	>128	>128	NA
Tobramycin	2–>128	>128	>128	NA
Vancomycin	≤0.5–>32	≤0.5	>32	87.3
Listeria monocytogenes (*n* = 25)				
Besifloxacin	0.06–0.25	0.12	0.12	NA
Moxifloxacin	0.12–0.5	0.25	0.5	NA
Ciprofloxacin	0.25–1	1	1	NA
Levofloxacin	0.25–2	0.5	1	NA
Azithromycin	0.25–1	0.5	1	NA
Tobramycin	≤0.06–0.25	0.12	0.12	NA
Penicillin	≤0.06–0.5	0.25	0.25	100.0
Staphylococcus aureus (*n* = 19; CIP-S; 21.1% MRSA)				
Besifloxacin	0.015–0.25	0.015	0.12	NA
Moxifloxacin	0.015–0.06	0.03	0.06	100.0
Gatifloxacin	0.03–1	0.06	0.25	94.7
Ciprofloxacin	0.12–0.5	0.25	0.5	100.0
Levofloxacin	0.06–2	0.12	0.25	94.7
Azithromycin	0.5–>8	1	>8	68.4
Tobramycin	0.12–8	0.5	1	94.7
Oxacillin	0.12–>8	0.25	>8	78.9
Staphylococcus aureus (*n* = 11; CIP-NS; 63.6% MRSA)				
Besifloxacin	0.03–4	0.5	4	NA
Moxifloxacin	0.06–>8	4	>8	9.1
Gatifloxacin	0.12–>8	4	>8	9.1
Ciprofloxacin	2–>8	>8	>8	0.0
Levofloxacin	0.25–>8	>8	>8	18.2
Azithromycin	0.5–>8	>8	>8	27.3
Tobramycin	0.25–>32	1	>32	54.5
Oxacillin	0.12–>8	>8	>8	36.4
Staphylococcus epidermidis (*n* = 9; CIP-S; 44.4% MRSE)				
Besifloxacin	0.015–0.03	0.03	NA	NA
Moxifloxacin	0.03–0.06	0.06	NA	100.0
Gatifloxacin	0.06–0.06	0.06	NA	100.0
Ciprofloxacin	0.12–0.12	0.12	NA	100.0
Levofloxacin	0.12–0.12	0.12	NA	100.0
Azithromycin	0.25–>8	>8	NA	33.3
Tobramycin	≤0.008–8	0.03	NA	88.9
Oxacillin	≤0.06–2	0.12	NA	55.6
Staphylococcus epidermidis (*n* = 6; CIP-NS; 83.3% MRSE)				
Besifloxacin	0.25–4	0.25	NA	NA
Moxifloxacin	1–>8	2	NA	0.0
Gatifloxacin	1–>8	1	NA	0.0
Ciprofloxacin	2–>8	>8	NA	0.0
Levofloxacin	2–>8	8	NA	0.0
Azithromycin	0.12–>8	>8	NA	16.7
Tobramycin	0.06–16	2	NA	83.3
Oxacillin	0.12–4	1	NA	16.7

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TABLE 1—*Continued*

Species or bacterial group (no. of isolates and phenotype[a]) and test drug	MIC (μg/ml)[b]			
	Range	50%	90%	%S[c]
Staphylococcus haemolyticus (*n* = 101)				
Besifloxacin	0.015–4	0.5	1	NA
Moxifloxacin	0.015–>8	1	8	39.6
Gatifloxacin	0.03–>8	2	8	40.6
Ciprofloxacin	0.06–>8	>8	>8	37.6
Levofloxacin	0.06–>8	4	>8	39.6
Azithromycin	0.25–>8	>8	>8	26.7
Tobramycin	0.015–>32	2	32	64.4
Oxacillin	≤0.06–>8	>8	>8	31.7
Staphylococcus hominis (*n* = 50)				
Besifloxacin	0.015–2	0.25	1	NA
Moxifloxacin	0.03–>8	1	4	34.0
Gatifloxacin	0.03–>8	1	4	32.0
Ciprofloxacin	0.06–>8	8	>8	30.0
Levofloxacin	0.06–>8	8	>8	30.0
Azithromycin	0.12–>8	>8	>8	16.0
Tobramycin	0.015–>32	16	32	32.0
Oxacillin	≤0.06–>8	>8	>8	16.0
Staphylococcus lugdunensis (*n* = 15)				
Besifloxacin	0.015–2	0.06	0.5	NA
Moxifloxacin	0.03–>8	0.12	2	73.3
Gatifloxacin	0.03–8	0.12	2	73.3
Ciprofloxacin	0.06–>8	0.12	>8	66.7
Levofloxacin	0.06–>8	0.25	>8	66.7
Azithromycin	0.25–>8	>8	>8	46.7
Tobramycin	0.03–>32	0.12	32	60.0
Oxacillin	≤0.06–>8	0.5	>8	60.0
Staphylococcus saprophyticus (*n* = 101)				
Besifloxacin	0.015–0.25	0.06	0.12	NA
Moxifloxacin	0.03–0.25	0.12	0.12	100.0
Gatifloxacin	0.03–0.25	0.12	0.25	100.0
Levofloxacin	0.06–0.5	0.5	0.5	100.0
Ciprofloxacin	0.06–0.5	0.25	0.5	100.0
Azithromycin	0.12–>8	1	>8	54.5
Tobramycin	≤0.008–32	0.015	0.06	99.0
Oxacillin	≤0.06–>8	0.5	1	9.9
Staphylococcus warneri (*n* = 50)				
Besifloxacin	0.015–2	0.06	1	NA
Moxifloxacin	0.015–>8	0.06	4	76.0
Gatifloxacin	0.03–>8	0.12	4	76.0
Ciprofloxacin	0.06–>8	0.25	>8	74.0
Levofloxacin	0.06–>8	0.12	>8	76.0
Azithromycin	0.12–>8	>8	>8	34.0
Tobramycin	0.015–>32	0.06	8	86.0
Oxacillin	≤0.06–>8	0.5	>8	46.0
Streptococcus agalactiae (*n* = 100)				
Besifloxacin	0.03–0.12	0.06	0.06	NA
Moxifloxacin	0.06–1	0.12	0.25	NA
Gatifloxacin	0.12–1	0.25	0.25	100.0
Ciprofloxacin	0.5–8	0.5	1	NA
Levofloxacin	0.25–4	0.5	1	98.0
Azithromycin	0.015–>8	0.06	>8	73.0
Tobramycin	8–>128	32	64	NA
Penicillin	≤0.015–0.06	0.03	0.06	100.0
Lancefield group C, F, and G streptococci (*n* = 50)				
Besifloxacin	0.015–0.25	0.03	0.06	NA
Moxifloxacin	0.03–1	0.12	0.12	NA
Gatifloxacin	0.06–2	0.12	0.25	98.0
Ciprofloxacin	0.12–>8	0.5	0.5	NA

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TABLE 1—*Continued*

Species or bacterial group (no. of isolates and phenotype[a]) and test drug	MIC (μg/ml)[b]			
	Range	50%	90%	%S[c]
Levofloxacin	0.12–8	0.5	0.5	98.0
Azithromycin	0.008–>8	0.06	>8	74.0
Tobramycin	2–32	8	16	NA
Penicillin	≤0.015–0.06	≤0.015	0.06	100.0
Streptococcus pneumoniae (*n* = 16; LVX-S)				
Besifloxacin	0.015–0.25	0.03	0.06	NA
Moxifloxacin	0.03–1	0.06	0.12	100.0
Ciprofloxacin	0.12–8	0.5	2	93.8
Levofloxacin	0.25–2	0.5	0.5	100
Azithromycin	≤0.03–>64	0.06	>64	62.5
Tobramycin	4–32	8	16	NA
Penicillin	≤0.06–2	≤0.06	0.5	100.0
Streptococcus pneumoniae (*n* = 85; LVX-NS)				
Besifloxacin	0.008–1	0.5	0.5	NA
Moxifloxacin	0.25–8	2	4	16.5
Ciprofloxacin	1–64	32	64	1.2
Levofloxacin	4–32	16	16	0
Azithromycin	≤0.03–>64	4	>64	36.5
Tobramycin	1–32	8	16	NA
Penicillin	≤0.06–2	0.12	2	100.0
Streptococcus pyogenes (*n* = 101)				
Besifloxacin	0.03–0.06	0.03	0.06	NA
Moxifloxacin	0.06–0.5	0.12	0.25	NA
Gatifloxacin	0.06–0.5	0.12	0.25	100.0
Ciprofloxacin	0.12–2	0.5	0.5	NA
Levofloxacin	0.25–2	0.5	0.5	100.0
Azithromycin	0.03–>8	0.06	8	85.1
Tobramycin	4–64	16	16	NA
Penicillin	≤0.015–0.06	≤0.015	≤0.015	100.0
Viridans streptococci[d] (*n* = 156)				
Besifloxacin	0.015–2	0.06	0.12	NA
Moxifloxacin	0.03–4	0.12	0.25	NA
Gatifloxacin	0.03–8	0.25	0.5	NA
Ciprofloxacin	0.12–>8	1	4	NA
Levofloxacin	0.12–>8	1	1	95.5
Azithromycin	0.008–>8	0.06	>8	53.2
Tobramycin	0.5–128	16	32	NA
Penicillin	≤0.015–>4	0.06	1	76.3

[a] VRE, vancomycin-intermediate and -resistant enterococci; MRSE, methicillin-resistant *S. epidermidis*; CIP-S, ciprofloxacin susceptible; CIP-NS, non-ciprofloxacin susceptible; LVX-S, levofloxacin susceptible; LVX-NS, non-levofloxacin susceptible.
[b] NA, not applicable.
[c] Percentage of susceptible isolates (9); breakpoints have not been defined for all agent-species combinations.
[d] The viridans streptococcal group consisted of 2 *Streptococcus anginosus*, 13 *S. bovis*, 7 *S. constellatus*, 28 *S. intermedius*, 51 *S. mitis*, 22 *S. oralis*, 2 *S. salivarius*, 17 *S. sanguinis*, and 14 other viridans group isolates.

cluded up to 46.8% azithromycin-resistant isolates, besifloxacin MIC_{90}s were 2- to 16-fold lower than those observed for other fluoroquinolones. In general, the activity of besifloxacin against streptococci was greater than that of the comparator fluoroquinolones as well as that of azithromycin and tobramycin.

Activity of besifloxacin against gram-negative aerobes. For three *Acinetobacter* spp. (which included 16 to 64% non-ciprofloxacin-susceptible isolates), the besifloxacin MIC_{90}s (16 to 32 μg/ml) were similar to or lower than the ciprofloxacin values, and for two of these species, the besifloxacin MIC_{90}s were two- to fourfold higher than the corresponding values for levofloxacin and moxifloxacin (Table 2).

For the *Enterobacteriaceae*, besifloxacin MIC_{90}s were identical to or lower than those of moxifloxacin for all species but *K. pneumoniae* (Table 2), where the besifloxacin MIC_{90} was twofold higher than that of moxifloxacin (4 versus 2 μg/ml, respectively). Ciprofloxacin and levofloxacin were generally the most active fluoroquinolones tested against the *Enterobacteriaceae*. However, a comparison of MIC_{90}s for species that included over 20% non-ciprofloxacin-susceptible isolates (*Enterobacter aerogenes*, *Morganella morganii*, and *Proteus mirabilis*) indicates that besifloxacin is especially active against fluoroquinolone-resistant isolates.

For the fastidious gram-negative species (*Haemophilus influenzae*, *Moraxella catarrhalis*, *Neisseria meningitidis*, and *Legionella pneumophila*), besifloxacin demonstrated potent activity equivalent to that of the fluoroquinolone comparators, with MIC_{90}s of 0.03 μg/ml or less for all test groups except non-ciprofloxacin-

TABLE 2. Activities of besifloxacin and comparators against gram-negative bacteria

Species (no. of isolates and phenotype[a]) and test drug	MIC (μg/ml)			
	Range	50%	90%	%S[b]
Acinetobacter baumanii (*n* = 53)				
Besifloxacin	0.015–64	16	32	NA
Moxifloxacin	0.03–128	8	32	NA
Ciprofloxacin	0.06–>128	64	>128	35.8
Levofloxacin	0.03–128	8	64	37.7
Imipenem	0.12–>8	2	>8	54.7
Tobramycin	0.12–>64	4	>64	60.4
Ceftazidime	2–>16	>16	>16	39.6
Acinetobacter calcoaceticus (*n* = 27)				
Besifloxacin	0.06–32	0.5	16	NA
Moxifloxacin	0.015–128	2	8	NA
Ciprofloxacin	0.03–>128	1	128	51.9
Levofloxacin	0.015–128	0.5	8	66.7
Imipenem	0.06–>8	0.25	1	92.6
Tobramycin	0.12–>64	1	>64	77.8
Ceftazidime	2–>16	4	>16	63.0
Acinetobacter lwoffii (*n* = 50)				
Besifloxacin	0.015–64	0.25	16	NA
Moxifloxacin	0.008–128	0.06	4	NA
Ciprofloxacin	0.004–>128	0.12	16	84.0
Levofloxacin	0.015–64	0.12	4	86.0
Imipenem	≤0.03–>8	0.06	0.5	94.0
Tobramycin	≤0.06–>64	0.25	8	86.0
Ceftazidime	0.5–>16	2	>16	76.0
Citrobacter freundii (*n* = 100)				
Besifloxacin	0.015–32	0.25	4	NA
Moxifloxacin	0.03–32	0.12	4	85.0
Ciprofloxacin	0.004–64	0.03	2	87.0
Levofloxacin	0.015–32	0.06	2	90.0
Imipenem	≤0.03–>8	0.25	0.5	99.0
Tobramycin	0.12–>64	0.5	8	87.0
Ceftazidime	0.06–>16	0.25	>16	78.0
Citrobacter koseri (*n* = 100)				
Besifloxacin	0.03–>8	0.06	0.25	NA
Moxifloxacin	0.015–>8	0.03	0.25	NA
Gatifloxacin	0.008–>8	0.015	0.12	99.0
Ciprofloxacin	0.004–>8	0.008	0.06	99.0
Levofloxacin	0.015–>8	0.03	0.12	99.0
Azithromycin	2–>8	8	>8	NA
Tobramycin	0.25–16	0.5	1	99.0
Ceftazidime	0.06–4	0.12	0.5	100.0
Enterobacter aerogenes (*n* = 50)				
Besifloxacin	0.03–16	0.25	8	NA
Moxifloxacin	0.03–64	0.12	16	80.0
Ciprofloxacin	0.008–64	0.015	16	80.0
Levofloxacin	0.03–64	0.06	16	80.0
Imipenem	0.12–4	0.5	1	100.0
Tobramycin	0.25–32	0.5	8	88.0
Ceftazidime	0.12–>16	2	>16	62.0
Enterobacter cloacae (*n* = 50)				
Besifloxacin	0.06–16	0.12	2	NA
Moxifloxacin	0.015–16	0.06	2	94.0
Ciprofloxacin	0.004–64	0.015	0.5	92.0
Levofloxacin	0.008–32	0.03	0.25	92.0
Imipenem	0.12–2	0.25	1	100.0
Tobramycin	0.25–32	0.5	8	88.0
Ceftazidime	0.12–>16	0.5	>16	70.0
Haemophilus influenzae (*n* = 15; CIP-S)				
Besifloxacin	0.008–0.25	0.015	0.03	NA
Moxifloxacin	0.015–0.5	0.015	0.06	100.0

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TABLE 2—*Continued*

Species (no. of isolates and phenotype[a]) and test drug	MIC (μg/ml)			
	Range	50%	90%	%S[b]
Ciprofloxacin	0.008–1	0.008	0.015	100.0
Levofloxacin	0.008–1	0.015	0.015	100.0
Azithromycin	0.12–2	2	2	100.0
Tobramycin	1–16	2	8	NA
Penicillin	0.25–>4	0.5	>4	NA
Haemophilus influenzae (n = 11; CIP-NS)				
Besifloxacin	0.015–2	2	2	NA
Moxifloxacin	1–16	8	16	9.1
Ciprofloxacin	2–64	16	64	0
Levofloxacin	1–32	8	16	27.3
Azithromycin	0.5–8	1	8	81.8
Tobramycin	1–16	4	8	NA
Penicillin	0.5–>4	1	2	NA
Klebsiella oxytoca (n = 50)				
Besifloxacin	0.06–8	0.12	1	NA
Moxifloxacin	0.03–8	0.06	2	NA
Gatifloxacin	0.015–8	0.03	0.5	92.0
Ciprofloxacin	0.008–>8	0.015	0.5	90.0
Levofloxacin	0.015–8	0.03	0.5	90.0
Azithromycin	8–>8	>8	>8	NA
Tobramycin	0.25–8	0.5	1	96.0
Ceftazidime	0.03–1	0.12	0.5	100.0
Klebsiella pneumoniae (n = 100)				
Besifloxacin	≤0.004–64	0.25	4	NA
Moxifloxacin	0.03–128	0.12	2	90.0
Ciprofloxacin	0.008–>128	0.03	2	89.0
Levofloxacin	≤0.004–128	0.06	2	91.0
Imipenem	≤0.03–2	0.25	0.5	100.0
Tobramycin	0.12–>64	0.25	1	93.0
Ceftazidime	≤0.03–>16	0.12	1	92.0
Legionella pneumophila (n = 50)				
Besifloxacin	0.015–0.06	0.03	0.03	NA
Moxifloxacin	0.015–0.06	0.03	0.06	NA
Gatifloxacin	0.015–0.06	0.03	0.06	NA
Ciprofloxacin	0.015–0.06	0.03	0.03	NA
Levofloxacin	0.015–0.06	0.03	0.03	NA
Azithromycin	0.03–1	0.12	1	NA
Tobramycin	0.25–4	1	2	NA
Moraxella catarrhalis (n = 101)				
Besifloxacin	0.015–0.12	0.03	0.03	NA
Moxifloxacin	0.015–0.12	0.03	0.03	NA
Gatifloxacin	0.008–0.25	0.015	0.015	NA
Ciprofloxacin	0.008–0.25	0.015	0.015	100.0
Levofloxacin	0.015–0.5	0.015	0.03	100.0
Azithromycin	0.015–0.06	0.03	0.03	100.0
Tobramycin	0.03–0.5	0.25	0.25	NA
Oxacillin	0.25–>8	4	8	NA
Morganella morganii (n = 51)				
Besifloxacin	0.03–>8	0.12	4	NA
Moxifloxacin	0.03–>8	0.25	>8	NA
Gatifloxacin	0.015–>8	0.12	>8	74.5
Ciprofloxacin	0.004–>8	0.015	>8	76.5
Levofloxacin	0.015–>8	0.06	8	76.5
Azithromycin	8–>8	>8	>8	NA
Tobramycin	0.25–32	1	4	90.2
Ceftazidime	0.03–>32	0.12	16	82.4
Neisseria meningitidis (n = 20)				
Besifloxacin	≤0.004–0.03	0.008	0.015	NA
Moxifloxacin	≤0.004–0.015	0.008	0.008	NA
Ciprofloxacin	≤0.002–0.015	0.004	0.004	100.0

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TABLE 2—*Continued*

Species (no. of isolates and phenotype[a]) and test drug	MIC (μg/ml)			
	Range	50%	90%	%S[b]
Levofloxacin	≤0.004–0.03	0.008	0.015	100.0
Azithromycin	≤0.03–0.12	0.06	0.06	100.0
Penicillin	≤0.06–0.25	≤0.06	0.12	50.0
Proteus mirabilis (*n* = 50)				
Besifloxacin	0.06–32	0.25	16	NA
Moxifloxacin	0.06–128	0.25	16	62.0
Ciprofloxacin	0.015–>128	0.03	32	64.0
Levofloxacin	0.015–32	0.03	8	76.0
Imipenem	0.06–4	1	2	100.0
Tobramycin	0.25–32	1	4	92.0
Ceftazidime	≤0.03–16	≤0.03	0.06	98.0
Proteus vulgaris (*n* = 50)				
Besifloxacin	0.06–1	0.12	0.25	NA
Moxifloxacin	0.06–2	0.25	0.5	100.0
Ciprofloxacin	0.008–0.12	0.015	0.03	100.0
Levofloxacin	0.015–0.25	0.03	0.06	100.0
Imipenem	0.25–4	1	4	100.0
Tobramycin	0.25–64	1	2	98.0
Ceftazidime	≤0.03–2	0.06	0.12	100.0
Pseudomonas aeruginosa (*n* = 105; CIP-S)				
Besifloxacin	0.5–8	1	4	NA
Moxifloxacin	0.5–16	2	4	NA
Ciprofloxacin	0.03–1	0.12	0.5	100.0
Levofloxacin	0.06–4	0.5	1	96.2
Imipenem	0.5–>8	2	2	95.2
Tobramycin	0.25–>64	0.5	1	97.1
Ceftazidime	0.5–>16	2	8	93.3
Pseudomonas aeruginosa (*n* = 96; CIP-NS)				
Besifloxacin	2–128	16	64	NA
Moxifloxacin	2–>128	64	>128	NA
Ciprofloxacin	2–>128	16	64	0
Levofloxacin	2–>128	16	64	1.0
Imipenem	0.25–>8	8	>8	44.8
Tobramycin	0.25–>64	1	>64	64.6
Ceftazidime	0.25–>16	4	>16	57.3
Serratia marcescens (*n* = 100)				
Besifloxacin	0.12–2	0.25	1	NA
Moxifloxacin	0.06–4	0.25	1	98.0
Ciprofloxacin	0.015–2	0.06	0.5	98.0
Levofloxacin	0.03–1	0.12	0.5	100.0
Imipenem	0.12–2	0.5	1	100.0
Tobramycin	0.25–>64	2	4	91.0
Ceftazidime	≤0.03–>16	0.06	0.5	93.0
Stenotrophomonas maltophilia (*n* = 48; LVX-S)				
Besifloxacin	0.25–8	1	4	NA
Moxifloxacin	0.03–1	0.5	1	NA
Ciprofloxacin	0.5–8	2	4	33.3
Levofloxacin	0.12–2	1	2	100.0
Imipenem	>8	>8	>8	0
Tobramycin	1–>64	16	>64	22.9
Ceftazidime	0.5–>16	16	>16	43.8
Stenotrophomonas maltophilia (LVX-NS; *n* = 52)				
Besifloxacin	1–>128	16	64	NA
Moxifloxacin	0.5–64	8	32	NA
Ciprofloxacin	4–128	32	64	0
Levofloxacin	4–64	8	32	0
Imipenem	2–>8	>8	>8	1.9
Tobramycin	0.25–>64	16	>64	38.5
Ceftazidime	0.5–>16	>16	>16	32.7

[a] CIP-S, ciprofloxacin susceptible; CIP-NS, non-ciprofloxacin susceptible; LVX-S, levofloxacin susceptible; LVX-NS, non-levofloxacin susceptible.
[b] Percentage of susceptible isolates (9); breakpoints have not been defined for all agent-species combinations. NA, not applicable.

susceptible *H. influenzae* (Table 2). Results from previous studies (4, 5, 17) were consistent with the current results, with all studies yielding besifloxacin MIC_{90}s of 0.06 μg/ml or less for ciprofloxacin-susceptible *H. influenzae*. For non-ciprofloxacin-susceptible isolates of *H. influenzae*, besifloxacin was 8- to 32-fold more active than the other fluoroquinolones.

Against ciprofloxacin-susceptible *Pseudomonas aeruginosa*, ciprofloxacin and levofloxacin were the most active fluoroquinolones (MIC_{90}s of 0.5 to 1 μg/ml), while both besifloxacin and moxifloxacin showed MIC_{90}s of 4 μg/ml (Table 2). However, against non-ciprofloxacin-susceptible *P. aeruginosa*, MIC_{90}s for besifloxacin, levofloxacin, and ciprofloxacin were 64 μg/ml, whereas the corresponding moxifloxacin MIC_{90} was at least fourfold higher (>128 μg/ml). For levofloxacin-susceptible and non-levofloxacin-susceptible *Stenotrophomonas maltophilia* isolates, the besifloxacin MIC_{90}s (4 and 64 μg/ml, respectively) were identical to those for ciprofloxacin and two- to fourfold higher than those for levofloxacin or moxifloxacin.

The overall activity of besifloxacin against gram-negative aerobic pathogens was generally similar to or two- to fourfold less than that of the other fluoroquinolones tested against fluoroquinolone-susceptible isolates. However, against non-ciprofloxacin-susceptible isolates, besifloxacin was more potent than or equal to the comparator fluoroquinolones.

Activity of besifloxacin against gram-positive and -negative anaerobes. For five of the six anaerobic species, besifloxacin MIC_{90}s were equal to or lower than those for the most active comparators, including clindamycin, metronidazole, and the other fluoroquinolones (Table 3). Against *Propionibacterium acnes*, the most active agent was clindamycin, with a 0.12 μg/ml MIC_{90}, while the corresponding besifloxacin and moxifloxacin values were twofold higher. Overall, besifloxacin and moxifloxacin were the most active agents tested against anaerobic bacteria.

Besifloxacin and drug-resistant bacteria. Recent results from the ocular TRUST project (2) indicated that 85% of methicillin-resistant *S. aureus* (MRSA) isolates are also resistant to fluoroquinolones. The improved potency of besifloxacin relative to that of the other fluoroquinolones as well as azithromycin and tobramycin against MRSA and non-ciprofloxacin-susceptible staphylococci should extend the agent's coverage. Although resistance to existing fluoroquinolones is at present still relatively rare among ocular *S. pneumoniae* and *H. influenzae* (2), the data presented here demonstrate that besifloxacin shows improved in vitro activity against fluoroquinolone-resistant isolates of these two major pathogens as well.

Previous genetic and biochemical studies demonstrated relatively balanced dual targeting of DNA gyrase and topoisomerase IV and lower in vitro spontaneous resistance rates for besifloxacin (7). Consistent with those results, the current study shows that for multiple gram-negative and gram-positive species, the differences in MIC between fluoroquinolone-susceptible and -resistant strains were less for besifloxacin than for the other members of this drug family. Further studies are warranted to more fully characterize how the potentially unique interactions between besifloxacin and both enzyme targets (7) may be related to the improved activity, reported herein, against a broad spectrum of clinical isolates that are resistant to earlier fluoroquinolones.

TABLE 3. Activities of besifloxacin and comparators against gram-positive and -negative anaerobic bacteria

Species (no. of isolates) and test drug	MIC (μg/ml)			
	Range	50%	90%	%S[a]
Bacteroides fragilis (n = 20)				
Besifloxacin	0.25–2	0.5	1	NA
Moxifloxacin	0.25–8	0.5	2	95.0
Gatifloxacin	1–16	2	4	NA
Clindamycin	0.5–>8	2	>8	65.0
Metronidazole	2–2	2	2	100
Clostridium perfringens (n = 21)				
Besifloxacin	0.12–0.25	0.25	0.25	NA
Moxifloxacin	0.25–0.5	0.5	0.5	100.0
Gatifloxacin	0.5–1	1	1	NA
Clindamycin	0.06–4	2	4	85.7
Metronidazole	1–4	2	4	100.0
Fusobacterium spp. (n = 21)				
Besifloxacin	0.12–8	0.25	1	NA
Moxifloxacin	0.25–>16	1	2	95.2
Gatifloxacin	0.5–>16	1	4	NA
Clindamycin	0.06–8	0.06	2	95.2
Metronidazole	≤0.12–2	0.25	1	100
Peptostreptococcus spp. (n = 52)				
Besifloxacin	0.06–2	0.25	0.5	NA
Moxifloxacin	0.25–4	0.25	0.5	98.1
Ciprofloxacin	0.5–>8	2	4	NA
Clindamycin	0.06–>8	0.25	>8	88.5
Metronidazole	≤0.03–>16	0.5	1	98.1
Prevotella spp. (n = 20)				
Besifloxacin	0.06–16	1	4	NA
Moxifloxacin	0.12–>16	4	8	45.0
Gatifloxacin	0.25–>16	8	16	NA
Clindamycin	≤0.03–>8	≤0.03	>8	85.0
Metronidazole	0.25–8	4	4	100.0
Propionibacterium acnes (n = 21)				
Besifloxacin	0.12–0.25	0.25	0.25	NA
Moxifloxacin	0.25–0.25	0.25	0.25	100.0
Gatifloxacin	0.25–0.5	0.25	0.5	NA
Clindamycin	≤0.03–2	0.06	0.12	100.0
Metronidazole	>16–>16	>16	>16	0

[a] Percentage of susceptible isolates (10); breakpoints have not been defined for all agent-species combinations. NA, not applicable.

Conclusions. Against 2,690 isolates, representing 34 aerobic and 6 anaerobic bacterial species, the in vitro activity of besifloxacin was generally equivalent or superior to that of existing agents used for topical treatment of ocular infections. The consistently improved activity profile of besifloxacin against gram-positive and gram-negative pathogens that were resistant to other fluoroquinolones was particularly notable. In conjunction with recently reported safety, efficacy, and pharmacokinetic results from clinical trials (14, 16, 18, 21), besifloxacin's broad-spectrum activity profile is appropriate for empirical treatment of bacterial infections.

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