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In Vitro Activities of DX-619 and Four Comparator Agents against 376 Anaerobic Bacterial Isolates

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The activity of DX-619 was evaluated against 376 anaerobic isolates using the reference CLSI agar dilution method. Overall, 90% of the strains were susceptible to DX-619 at ≤1 μg/ml. It was more active than the other four compounds tested except for meropenem, which showed virtually identical overall activity.

Resistance to antimicrobial agents has been observed in many if not most clinically significant pathogenic bacteria and is increasing in prevalence. New classes of antimicrobial agents and modifications of existing agents, as well as of methods of blocking bacterial resistance mechanisms, are essential for improving activity against these resistant organisms. DX-619 is a newly developed des-F(6)-quinolone (Fig. 1) that has been shown to be effective against multiresistant gram-positive bacteria including methicillin-, ciprofloxacin-, and vancomycin-resistant *Staphylococcus aureus*, ciprofloxacin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant enterococci (1, 4). It is currently under development for use in gram-positive infections. Garenoxacin, another desfluoroquinolone, has shown good broad-spectrum activity against gram-positive and gram-negative aerobes and anaerobes (3, 5, 8). This study compared the activity of DX-619 and that of four comparator agents (amoxicillin-clavulanate, linezolid, meropenem, and moxifloxacin, chosen from different classes of antimicrobials that are effective against anaerobes) against 376 strains of anaerobic bacteria.

The bacteria included in this study were recent isolates from the Greater Los Angeles Veterans Administration Healthcare Center. Bacteria were identified according to established procedures (6), supplemented in a number of cases by 16S rRNA sequence analysis. Most of the organisms studied are involved in a great variety of infections. MICs with regard to anaerobes were determined by the Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards)-approved Wadsworth agar dilution technique (2). A suspension of colonies taken from 48-h blood agar plates was used to achieve a final inoculum of 10^5 CFU/spot. The basal medium was brucella base laked-blood agar (Anaerobe Systems, Morgan Hill, CA) with hemin, vitamin K_1, and 5% laked sheep blood, supplemented with pyruvic acid (1% final concentration) for the growth of *Bilophila wadsworthia* and formic and fumaric acids (0.3%/0.3%) for *Sutterella wadsworthensis*. Plates were incubated in an anaerobic chamber (Anaerobe Systems) for 48 h at 37°C. MICs were defined as the lowest concentration of antimicrobial agent resulting in no growth or a marked change in the appearance of growth compared to the control plate, as described in the CLSI protocol. Triphenyltetrazolium chloride was used as an aid in interpreting the growth endpoints of *Bilophila wadsworthia* (10). Reference strains of *Bacteroides fragilis* (ATCC 25285), *Bacteroides thetaiotaomicron* (ATCC 29741), and *Eggerthella lenta* (ATCC 43055) were used as controls in each test. The antimicrobial agents tested were obtained as powders from the following companies: amoxicillin, Sigma, St. Louis, MO.; clavulanate, GlaxoSmithKline, King of Prussia, PA; DX-619, Daiichi, Tokyo, Japan; linezolid, Pfizer, Groton, CT; meropenem, AstraZeneca, Wilmington, Del.; moxifloxacin, Bayer, West Haven, CT.

The ranges and the MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of isolates were inhibited are presented in Table 1. DX-619 demonstrated potent activity against a broad spectrum of gram-negative and gram-positive anaerobes, inhibiting 340 of 376 strains (90%) at ≤1 μg/ml; MICs ranged from ≤0.12 μg/ml to 8 μg/ml. On a weight basis (no breakpoint has been set as yet), DX-619 was comparable to meropenem in overall activity and at least three dilutions more active than the other three compounds tested. DX-619 and meropenem had the same MIC_{90}s against the *B. fragilis* group of organisms (MIC_{90}, 2 μg/ml), *Fusobacterium* species (MIC_{90}, 0.25 μg/ml), and *Porphyromonas* species (MIC_{90}, 0.12 μg/ml). Within the *B. fragilis* group DX-619 was most effective against *Bacteroides caccae*, *Bacteroides distasonis*/*merdae*, *B. fragilis*, and an unspeciated group of 8 *B. fragilis* group strains (MIC_{90}, 0.5 μg/ml) and was least active against *Bacteroides vulgatus* (MIC_{90}, 8 μg/ml). Meropenem showed strongest activity (MIC_{90}, 0.5 μg/ml) versus *B. fragilis*, *Bacteroides stercoris*, and *Bacteroides uniformis*. Amoxicillin-clavulanate, linezolid, and moxifloxacin had over-

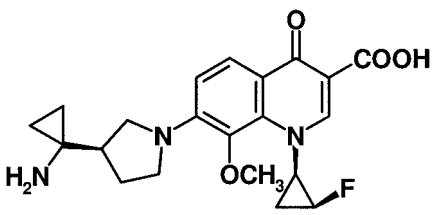

FIG. 1. Chemical structure of DX-619.

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TABLE 1. In vitro activities of DX-619 and four other antimicrobial agents against 376 anaerobic bacteria

Organism (no. of strains) and antimicrobial agent	MIC (μg/ml)		
	50%	90%	Range
Bacteroides caccae (10)			
DX-619	≤0.12	0.5	≤0.12–0.50
Amoxicillin-clavulanate	0.5	8	0.25–32
Linezolid	4	4	2–8
Meropenem	0.25	1	≤0.12–2
Moxifloxacin	2	2	0.50–4
Bacteroides distasonis/*merdae* (11)			
DX–619	≤0.12	0.5	≤0.12–1
Amoxicillin-clavulanate	8	32	2–32
Linezolid	4	8	4–8
Meropenem	1	2	0.25–16
Moxifloxacin	0.5	8	≤0.12–16
Bacteroides fragilis (41)			
DX–619	≤0.12	0.5	≤0.12–2
Amoxicillin-clavulanate	0.5	4	0.25–16
Linezolid	4	4	2–8
Meropenem	≤0.12	0.5	≤0.12–8
Moxifloxacin	0.5	8	0.25–32
Bacteroides ovatus (10)			
DX–619	≤0.12	2	≤0.12–2
Amoxicillin-clavulanate	0.5	8	0.50–32
Linezolid	4	4	2–4
Meropenem	0.25	1	≤0.12–2
Moxifloxacin	2	8	1–32
Bacteroides stercoris (10)			
DX–619	≤0.12	1	≤0.12–4
Amoxicillin-clavulanate	0.5	4	0.50–8
Linezolid	4	8	1–16
Meropenem	0.25	0.5	≤0.12–2
Moxifloxacin	1	1	0.50–64
Bacteroides thetaiotaomicron (39)			
DX–619	0.25	2	≤0.12–4
Amoxicillin-clavulanate	1	16	0.50–32
Linezolid	4	8	4–16
Meropenem	0.5	2	≤0.12–4
Moxifloxacin	2	32	0.50–64
Bacteroides uniformis (12)			
DX–619	0.25	2	≤0.12–2
Amoxicillin-clavulanate	1	8	0.50–8
Linezolid	2	4	2–4
Meropenem	0.25	0.5	≤0.12–1
Moxifloxacin	2	32	1–64
Bacteroides vulgatus (11)			
DX–619	4	8	≤0.12–8
Amoxicillin-clavulanate	4	16	1–16
Linezolid	2	4	1–8
Meropenem	0.5	1	0.25–2
Moxifloxacin	32	128	1–128
Bacteroides fragilis group species[a] (10)			
DX–619	≤0.12	0.25	≤0.12–0.50
Amoxicillin-clavulanate	2	4	≤0.12–32
Linezolid	2	4	1–8
Meropenem	0.25	1	≤0.12–4
Moxifloxacin	0.5	1	0.25–1
Bilophila wadsworthia (16)			
DX-619	1	2	0.50–2
Amoxicillin-clavulanate	2	16	1–64
Linezolid	16	32	8–32
Meropenem	≤0.12	≤0.12	≤0.12
Moxifloxacin	0.25	0.5	≤0.12–0.50
Campylobacter gracilis (11)			
DX–619	0.25	1	≤0.12–1
Amoxicillin-clavulanate	≤0.12	2	≤0.12–>64
Linezolid	16	32	4–64
Meropenem	≤0.12	≤0.12	≤0.12
Moxifloxacin	0.25	64	≤0.12–64
Fusobacterium species[b] (35)			
DX-619	≤0.12	0.25	≤0.12–0.50
Amoxicillin-clavulanate	≤0.12	4	≤0.12–64
Linezolid	0.5	1	0.25–2
Meropenem	≤0.12	0.25	≤0.12–1
Moxifloxacin	1	4	≤0.12–8
Porphyromonas species[c] (23)			
DX-619	≤0.12	≤0.12	≤0.12
Amoxicillin-clavulanate	≤0.12	≤0.12	≤0.12
Linezolid	1	2	0.50–2
Meropenem	≤0.12	≤0.12	≤0.12
Moxifloxacin	0.5	2	≤0.12–16
Prevotella species[d] (28)			
DX-619	0.25	2	≤0.12–8
Amoxicillin-clavulanate	0.25	4	≤0.12–4
Linezolid	1	4	0.25–4
Meropenem	≤0.12	≤0.12	≤0.12–0.25
Moxifloxacin	0.5	8	0.25–16
Sutterella wadsworthensis (11)			
DX-619	2	2	≤0.12–2
Amoxicillin-clavulanate	2	4	0.25–4
Linezolid	128	>128	32–>128
Meropenem	≤0.12	≤0.12	≤0.12
Moxifloxacin	0.25	1	≤0.12–1
Clostridium species[e] (39)			
DX-619	≤0.12	0.25	≤0.12–0.25
Amoxicillin-clavulanate	0.25	2	≤0.12–8
Linezolid	2	4	0.50–8
Meropenem	0.5	4	≤0.12–4
Moxifloxacin	1	8	≤0.12–8
Anaerobic non-spore-forming gram-positive rods[f] (31)			
DX-619	≤0.12	2	≤0.12–2
Amoxicillin-clavulanate	0.25	1	≤0.12–2
Linezolid	1	2	0.25–8
Meropenem	≤0.12	1	≤0.12–4
Moxifloxacin	1	4	≤0.12–8
Anaerobic gram-positive cocci[g] (28)			
DX-619	≤0.12	0.25	≤0.12–0.25
Amoxicillin-clavulanate	≤0.12	0.5	≤0.12–0.50
Linezolid	1	2	0.25–2
Meropenem	≤0.12	0.5	≤0.12–0.50
Moxifloxacin	0.25	4	≤0.12–8
Total for all strains (376)			
Amoxicillin-clavulanate	0.5	8	≤0.12–>64
DX–619	≤0.12	1	≤0.12–8
Linezolid	2	8	0.25–>128
Moxifloxacin	1	8	≤0.12–128
Meropenem	≤0.12	1	≤0.12–16

[a] *Bacteroides fragilis* group species (three strains), *Bacteroides nordii* (three strains), and *Bacteroides salyersiae* (four strains).
[b] *Fusobacterium gonidiaformans* (2 strains), *Fusobacterium mortiferum* (7 strains), *Fusobacterium necrogenes* (1 strain), *Fusobacterium necrophorum* (10 strains), *Fusobacterium nucleatum* (13 strains), and *Fusobacterium varium* (2 strains).
[c] *Porphyromonas asaccharolytica* (six strains), *Porphyromonas endodontalis* (five strains), *Porphyromonas gingivalis* (five strains), *Porphyromonas somerae* (five strains), and *Porphyromonas uenonis* (two strains).
[d] *Prevotella bivia* (five strains), *Prevotella buccae* (one strain), *Prevotella corporis* (one strain), *Prevotella dentalis* (one strain), *Prevotella denticola* (two strains), *Prevotella disiens* (four strains), *Prevotella intermedia* (two strains), *Prevotella intermedia-nigrescens* (three strains), *Prevotella loescheii* (one strain), *Prevotella melaninogenica* (four strains), *Prevotella oralis* (one strain), *Prevotella oris* (two strains), and *Prevotella* spp. (one strain).
[e] *Clostridium bartlettii* (one strain), *Clostridium beijerinckii* (one strain), *Clostridium bifermentans* (one strain), *Clostridium bolteae* (two strains), *Clostridium butyricum* (one strain), *Clostridium clostridioforme* (three strains), *Clostridium difficile* (six strains), *Clostridium disporicum* (one strain), *Clostridium glycolicum* (two strains), *Clostridium hastiforme* (one strain), *Clostridium hathewayi* (two strains), *Clostridium innocuum* (two strains), *Clostridium leptum* (one strain), *Clostridium paraputrificum* (one strain), *Clostridium perfringens* (five strains), *Clostridium ramosum* (three strains), *Clostridium sordellii* (three strains), *Clostridium sporogenes* (one strain), *Clostridium subterminale* (one strain), and *Clostridium tertium* (one strain).
[f] *Actinomyces europaeus* (one strain), *Actinomyces israelii* (three strains), *Actinomyces naeslundii* (two strains), *Actinomyces odontolyticus* (two strains), *Actinomyces viscosus* (one strain), *Atopobium minutum* (two strains), *Bifidobacterium adolescentis* (one strain), *Bifidobacterium breve* (one strain), *Bifidobacterium dentium* (one strain), *Bifidobacterium pseudocatenulatum* (one strain), *Bifidobacterium* species (one strain), *Collinsella aerofaciens* (two strains), *Eggerthella lenta* (two strains), *Eubacterium biforme* (one strain), *Eubacterium limosum* (one strain), *Lactobacillus acidophilus* (one strain), *Lactobacillus catenaforme* (one strain), *Lactobactillus fermentum* (one strain), *Lactobacillus jensenii* (one strain), *Propionibacterium acnes* (two strains), *Propionibacterium avidum* (two strains), and *Propionibacterium propionicus* (one strain).
[g] *Anaerococcus prevotii* (three strains), *Anaerococcus tetradius* (two strains), *Finegoldia magna* (six strains *Peptostreptococcus anaerobius* (six strains), *Peptostreptococcus micros* (five strains), *Peptoniphilus asaccharolyticus* (one strain), *Peptoniphilus harei* (one strain), *Ruminococcus gnavus* (two strains), *Ruminococcus lactaris* (one strain), and *Ruminococcus productus* (one strain).

all MIC_{90}s of 16, 8, and 32 μg/ml, respectively, against the *B. fragilis* group. Twenty-two strains of *B. fragilis* group organisms that were resistant to moxifloxacin (MICs, 16 to 64 μg/ml) had MICs of 1 to 4 μg/ml with DX-619 and ≤0.12 to 2 μg/ml with meropenem. Two strains of *B. vulgatus* with moxifloxacin MICs of 128 μg/ml were more susceptible to DX-619 (MICs, 8 μg/ml) and meropenem (MICs, 0.5 and 1 μg/ml). Amoxicillin-clavulanate was equivalent to DX-619 and meropenem when tested against *Porphyromonas* species (MIC_{90}, ≤0.12 μg/ml); the MIC_{90}s for linezolid and moxifloxacin were 2 μg/ml. DX-619 inhibited 100% of *Bilophila wadsworthia*, *Campylobacter gracilis*, and *Sutterella wadsworthensis* strains and 93% of *Prevotella* strains at 2 μg/ml. However, meropenem showed the strongest activity against these organisms (all strains inhibited by ≤0.25 μg/ml). Moxifloxacin was also effective against *Bilophila wadsworthia* (MIC_{90}, 0.5 μg/ml) and *Sutterella wadsworthensis* (MIC_{90}, 1 μg/ml).

Among gram-positive anaerobic organisms, DX-619 was the most potent antimicrobial agent tested. DX-619 was the most effective agent against clostridia; at a concentration of 0.25 μg/ml, all 39 strains were inhibited. MIC_{90}s for amoxicillin-clavulanate, linezolid, meropenem, and moxifloxacin against clostridia were 2, 4, 4, and 8 μg/ml, respectively. DX-619 was 2 to 4 dilutions more active than the other compounds tested against *Clostridium difficile* ($n = 6$). Against non-spore-forming gram-positive rods, amoxicillin-clavulanate, DX-619, and meropenem demonstrated very similar activities; MICs ranged from ≤0.12 to 4 μg/ml. Linezolid and moxifloxacin were less potent than the other agents, with an MIC_{50} of ≤1 μg/ml, but inhibited all strains of non-spore-forming gram-positive rods at ≤8 μg/ml. DX-619 had the most potent activity against anaerobic gram-positive cocci (MIC_{90}, ≤0.25 μg/ml); amoxicillin-clavulanate, linezolid, meropenem, and moxifloxacin had MIC_{90}s of 0.5, 2, 0.5, and 4 μg/ml, respectively.

Overall, DX-619 performed comparably to meropenem and was more active than amoxicillin-clavulanate, linezolid, and moxifloxacin against the diverse group of anaerobic organisms tested. Most notably, DX-619 inhibited all clostridia at an MIC of ≤0.25 μg/ml, besting all the other drugs by 4 to 5 dilutions. These results are in accordance with and perhaps somewhat surpass (in the case of *Clostridium* and *Fusobacterium* spp.) those obtained from studies of garenoxacin, another desfluoroquinolone. Animal studies of garenoxacin (7, 9) found it to produce less joint cartilage damage than the other quinolones tested. Additional studies are needed to assess the clinical utility and possible toxicity of drugs, such as DX-619, which show good in vitro activity against clinically important gram-positive and gram-negative anaerobic organisms.

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REFERENCES

1. **Bogdanovich, T., D. Esel, L. M. Kelly, B. Bozdogan, K. Credito, G. Lin, K. Smith, L. M. Ednie, D. B. Hoellman, and P. C. Appelbaum.** 2005. Antistaphylococcal activity of DX-619, a new des-F(6)-quinolone, compared to those of other agents. Antimicrob. Agents Chemother. **49:**3325–3333.
2. **Clinical and Laboratory Standards Institute.** 2004. Methods for antimicrobial susceptibility testing of anaerobic bacteria: approved standard, 6th ed.; document M11–A6. Clinical and Laboratory Standards Institute, Wayne, Pa.
3. **Credito, K. L., M. R. Jacobs, and P. C. Appelbaum.** 2003. Time-kill studies of the antianaerobe activity of garenoxacin compared with those of nine other agents. Antimicrob. Agents Chemother. **47:**1399–1402.
4. **Fujikawa, K., M. Chiba, M. Tanaka, and K. Sato.** 2005. In vitro antibacterial activity of DX-619, a novel des-fluoro(6) quinolone. Antimicrob. Agents Chemother. **49:**3040–3045.
5. **Hecht, D., and J. R. Osmolski.** 2002. Activities of garenoxacin (BMS-284756) and other agents against anaerobic clinical isolates. Antimicrob. Agents Chemother. **47:**910–916.
6. **Jousimies-Somer, H. R., P. Summanen, D. M. Citron, E. J. Baron, H. M. Wexler, and S. M. Finegold.** 2002. Wadsworth-KTL anaerobic bacteriology manual, 6th ed. Star Publishing Co., Belmont, Calif.
7. **Kappel, E. M., M. Shakibaei, A. Bello, and R. Stahlmann.** 2002. Effects of the des-F(6)-quinolone garenoxacin (BMS-284756), in comparison to those of ciprofloxacin and ofloxacin, on joint cartilage in immature rats. Antimicrob Agents Chemother. **46:**3320–3322.
8. **Liebetrau, A., A. C. Rodloff, J. Behra-Miellet, and L. Dubreuil.** 2003. In vitro activities of a new des-fluoro(6) quinolone, garenoxacin, against clinical anaerobic bacteria. Antimicrob. Agents Chemother. **47:**3667–3671.
9. **Nagai, A., M. Miyazaki, T. Morita, S. Furubo, K. Kizawa, H. Fukumoto, T. Sanzen, H. Hayakawa, and Y. Kawamura.** 2002. Comparative articular toxicity of garenoxacin, a novel quinolone antimicrobial agent, in juvenile beagle dogs. J. Toxicol. Sci. **27:**219–228.
10. **Summanen, P., H. M. Wexler, and S. M. Finegold.** 1992. Antimicrobial susceptibility testing of *Bilophila wadsworthia* by using triphenyltetrazolium chloride to facilitate endpoint determination. Antimicrob. Agents Chemother. **36:**1658–1664.