

## In Vitro Antianaerobic Activity of DX-619, a New Des-Fluoro(6) Quinolone

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**The in vitro activity of DX-619, a new des-F(6) quinolone, against anaerobic bacteria was evaluated. DX-619 showed potent activity against *Bacteroides*, *Prevotella*, *Fusobacterium*, *Micromonas*, *Actinomyces*, and *Clostridium* spp., with MIC<sub>50</sub>s/MIC<sub>90</sub>s of ≤0.03 to 0.25/≤0.03 to 1 μg/ml, respectively. DX-619 was also active against imipenem-resistant *Bacteroides* spp., with MIC<sub>50</sub>s/MIC<sub>90</sub>s of 0.25/1 μg/ml, respectively.**

Older fluoroquinolones, such as ciprofloxacin and ofloxacin, are inactive or only partially active against anaerobic bacteria (7, 11, 12, 15, 20, 23, 25, 26). Newer quinolones, such as sparfloxacin and levofloxacin (LVX), have improved in vitro activities against anaerobic bacteria but still have limited activities against certain gram-positive and gram-negative anaerobic bacilli (3, 7, 8, 11, 12, 15, 23, 25). Some of the newer fluoroquinolones are much more active to both gram-positive and gram-negative anaerobic bacteria (3, 8, 11, 12, 15, 20, 26). DX-619 is a new derivative of quinolone, defined as a des-F(6) quinolone that lacks the six-position fluorine characteristic of the previous generation of fluoroquinolones. It has potent antibacterial activity against multidrug-resistant, gram-positive aerobic bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci (2, 5). Other compounds of this new type of quinolone showed potent activities against gram-positive and gram-negative aerobic and anaerobic bacteria (23, 25). There have been few reports on the antibacterial activity of DX-619 against anaerobic bacteria (16). We evaluated the in vitro activity of DX-619 against anaerobic gram-positive and gram-negative species in comparison with that of several reference agents.

For the investigation of the anaerobic antibacterial spectrum, a total of 71 gram-positive and gram-negative reference strains (64 species in 25 genera) of anaerobic bacteria and some fastidious microaerophilic anaerobes were examined. Those reference strains include strains obtained from the American Type Culture Collection (ATCC; Virginia), the German Collection of Microorganisms and Cell Cultures (DSMZ; Braunschweig, Germany), the Japan Collection of Microorganisms (JCM; Saitama, Japan), the National Collection of Type Cultures (NCTC; Wiltshire, United Kingdom), and VPI (Virginia Polytechnic Institute and State University, Virginia) and some characteristic clinical strains belong to GAI (the culture collection of our laboratory). A total of 268 clinical strains isolated from various sources (including intra-abdominal infection, head and neck space infection, pleuropulmonary infection, and soft-tissue infection) between 1994 and 2004

were also studied. Isolates were identified by standard criteria (9, 10, 22). In addition, the activities of DX-619 against 11 stock strains of the imipenem (IPM)-resistant *Bacteroides fragilis* group (10 *Bacteroids fragilis* and 1 *B. thetaiotaomicron* strain) were also studied.

The antibacterial agents used in this study were obtained as powders of known potency from their respective manufacturers. DX-619 was obtained from Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan. We used four antianaerobic agents, IPM (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan), cefmetazole (CMZ), clindamycin (CLI), and metronidazole (MNZ) (Sigma-Aldrich Japan, Tokyo, Japan), as the reference agents for DX-619. One of the most popular fluoroquinolones, LVX (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), and a popular cephalosporin for outpatient therapy, ceftriaxone (Sigma-Aldrich Japan, Tokyo, Japan), were also examined. The susceptibility of MNZ was examined only for reference strains.

The MICs were determined by an agar dilution method in accordance with CLSI (NCCLS) document M11-A5 (17). Brucella HK agar (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) supplemented with 5% laked sheep blood was used as the test medium. The test strains (10<sup>5</sup> CFU/spot) were inoculated and incubated at 35°C in an anaerobic chamber (82% N<sub>2</sub>, 10% CO<sub>2</sub>, 8% H<sub>2</sub>). *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were used as quality control strains.

The results of the susceptibility test on the reference strains are listed in Tables 1 and 2. Overall, DX-619 showed potent activities against both gram-positive and -negative anaerobic reference strains. Most strains were inhibited at 0.5 μg/ml or less of this agent, including beta-lactam-resistant strains (*B. fragilis* GAI 0558, 7955, and 10150). DX-619 was more active than CMZ, ceftriaxone, LVX, and MNZ. Also, DX-619 was more active than CLI and almost as active as or more active than IPM against gram-positive cocci and *Clostridium* spp. In gram-negative organisms, DX-619 was slightly less active than IPM and CLI against *Prevotella* spp. It was, however, more active than IPM and CLI against the *Bacteroides fragilis* group.

Table 3 shows the in vitro activities of DX-619 and reference agents against clinical strains frequently isolated in anaerobic infection. The results are expressed as MIC<sub>50</sub>s, MIC<sub>90</sub>s, and percentages of isolates susceptible at the indicated drug concentrations. Against three species of the *B. fragilis* group and

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TABLE 1. Antimicrobial activities of DX-619 and other reference compounds against gram-positive anaerobic bacteria and facultative anaerobic bacteria

Organism	MIC ( $\mu\text{g/ml}$ )						
	DX-619	Ceftriaxone	Cefmetazole	Imipenem	Clindamycin	Levofloxacin	Metronidazole
<i>Anaerococcus prevotii</i> ATCC 9321	0.125	1	0.5	0.25	0.125	4	2
<i>Atopobium parvulum</i> VPI 0546	0.125	0.5	2	0.25	1	0.5	2
<i>Finegoldia magna</i> ATCC 29328	$\leq 0.03$	4	1	2	2	0.25	0.5
<i>Micromonas micros</i> VPI 5464-1	$\leq 0.03$	0.25	0.25	0.25	0.25	0.5	0.5
<i>Peptoniphilus asaccharolyticus</i> WAL3218	0.06	0.25	0.25	0.25	>128	4	1
<i>Peptoniphilus indolicus</i> GAI0915	0.06	$\leq 0.03$	0.06	0.5	32	4	0.5
<i>Peptostreptococcus anaerobius</i> ATCC 27337	$\leq 0.03$	0.5	0.5	0.06	0.25	0.25	0.125
<i>Gemella morbillorum</i> ATCC 27824	0.06	$\leq 0.03$	0.25	$\leq 0.03$	0.06	1	>16
<i>Staphylococcus saccharolyticus</i> ATCC 14953	$\leq 0.03$	0.5	0.5	$\leq 0.03$	0.125	0.5	>16
<i>Streptococcus constellatus</i> ATCC 27923	$\leq 0.03$	0.5	2	0.125	0.25	1	>16
<i>Streptococcus intermedius</i> ATCC 27335	$\leq 0.03$	0.25	2	0.5	0.25	1	>16
<i>Clostridium bif fermentans</i> NCTC06800	0.06	0.25	0.25	0.125	1	1	ND <sup>a</sup>
<i>Clostridium clostridioforme</i> NCTC11224	0.06	4	16	0.5	0.25	16	0.03
<i>Clostridium difficile</i> GAI10029	0.125	32	32	4	>128	4	0.25
<i>Clostridium paraputrificum</i> ATCC 25780	0.06	4	0.5	0.5	8	1	ND
<i>Clostridium perfringens</i> ATCC 13124	$\leq 0.03$	0.125	0.125	0.125	0.06	0.25	0.5
<i>Clostridium putrificum</i> ATCC 25784	$\leq 0.03$	4	0.25	0.125	32	0.25	ND
<i>Clostridium septicum</i> ATCC 12464	0.06	1	0.125	$\leq 0.03$	$\leq 0.03$	0.125	0.25
<i>Clostridium sordellii</i> NCTC06929	0.06	0.25	0.25	0.125	1	1	ND
<i>Clostridium sordellii</i> NCTC08780	0.06	0.25	0.5	0.125	1	1	ND
<i>Clostridium ramosum</i> ATCC 25582	0.25	0.5	4	0.25	8	8	1
<i>Actinomyces odontolyticus</i> GAI91002	0.25	0.25	0.5	2	0.25	8	16
<i>Bifidobacterium adolescentis</i> ATCC 15703	0.5	0.125	0.25	1	$\leq 0.03$	1	>16
<i>Bifidobacterium bifidum</i> JCM1255	0.5	1	0.25	0.25	$\leq 0.03$	8	4
<i>Bifidobacterium breve</i> ATCC 15700	0.5	2	4	2	$\leq 0.03$	8	16
<i>Bifidobacterium longum</i> subsp. <i>longum</i> ATCC 15707	1	4	4	8	$\leq 0.03$	4	8
<i>Bifidobacterium pseudolongum</i> ATCC 25526	1	0.5	0.5	8	$\leq 0.03$	8	>16
<i>Eggerthella lenta</i> ATCC 25559	0.125	>128	16	8	0.25	0.5	0.25
<i>Eggerthella lenta</i> ATCC 43055	0.125	>128	16	4	0.125	0.5	ND
<i>Propionibacterium acnes</i> ATCC 11828	0.125	0.5	1	0.125	0.125	0.5	>16
<i>Propionibacterium granulosum</i> ATCC 25564	0.06	0.125	2	0.125	$\leq 0.03$	0.25	>16
<i>Lactobacillus acidophilus</i> JCM1132	0.125	1	8	0.125	1	32	>16
<i>Lactobacillus brevis</i> subsp. <i>brevis</i> JCM1059	0.06	8	64	$\leq 0.03$	$\leq 0.03$	8	>16
<i>Lactobacillus casei</i> subsp. <i>casei</i> JCM1134	$\leq 0.03$	4	128	1	2	1	>16
<i>Lactobacillus fermentum</i> JCM1173	0.125	64	128	$\leq 0.03$	$\leq 0.03$	8	>16
<i>Lactobacillus plantarum</i> JCM1149	0.250	0.125	>128	0.06	0.25	8	>16
<i>Lactobacillus reuteri</i> JCM1112	0.06	16	>128	0.06	$\leq 0.03$	16	>16
<i>Lactobacillus salivarius</i> subsp. <i>salivarius</i> JCM1231	$\leq 0.03$	4	32	1	0.06	1	>16

<sup>a</sup> ND, not done.

*Fusobacterium* spp., the most active agent was DX-619, with MIC<sub>90</sub>s of 0.25 to 1  $\mu\text{g/ml}$ . For the *B. fragilis* group, IPM was the second-most-active agent, with MIC<sub>90</sub>s of 1 to 4  $\mu\text{g/ml}$ . Although CLI still showed strong activity against 68% of *B. fragilis* isolates, with a MIC<sub>50</sub> of 1  $\mu\text{g/ml}$ , the MIC<sub>50</sub> of DX-619 (0.125  $\mu\text{g/ml}$ ) was threefold lower than that of CLI. In *Fusobacterium* spp., overall, the strains examined were sensitive to all six test agents and quite sensitive to DX-619. Against *Prevotella*, IPM and CLI showed superior activities. DX-619 was the third-most-active agent in this group but also showed potent activity, inhibiting all strains with a concentration of  $\leq 1$   $\mu\text{g/ml}$ .

DX-619 was quite active for gram-positive organisms. Similarly to *Fusobacterium* strains, strains of *Micromonas micros* and *Propionibacterium acnes* were sensitive to six test agents, including DX-619. DX-619 inhibited those strains at  $\leq 0.03$   $\mu\text{g/ml}$  (*M. micros*) and 0.125  $\mu\text{g/ml}$  (*P. acnes*). The *Actinomyces* spp. included species variously susceptible to beta-lactams, but all strains were sensitive to DX-619 and CLI, with MIC<sub>90</sub>s of 1

$\mu\text{g/ml}$ . As for clostridia, *Clostridium perfringens* and other *Clostridium* spp. had similar susceptibilities and most of the strains tested were susceptible to all six agents. Against these two groups, DX-619, IPM, and CMZ had potent activities (from highest to lowest, in that order), with MIC<sub>90</sub>s of  $\leq 0.25$   $\mu\text{g/ml}$ . On the other hand, most of the agents, including IPM and CMZ, were less active against *Clostridium difficile*. The MIC<sub>90</sub>s for IPM and CMZ were 16 and 64  $\mu\text{g/ml}$ , respectively, but DX-619 was very active against *C. difficile* and inhibited all *C. difficile* isolates at a concentration of 2  $\mu\text{g/ml}$  (Fig. 1).

The IPM-resistant *B. fragilis* group strains (11 strains) and LVX-resistant anaerobic gram-positive cocci (14 strains) were also examined (Table 4). All but one strain (90.9%) of the IPM-resistant *B. fragilis* group were inhibited by  $\leq 1$   $\mu\text{g/ml}$  of DX-619. The MIC<sub>90</sub>s for the other agents were 16  $\mu\text{g/ml}$  for LVX and equal to or more than 128  $\mu\text{g/ml}$  for four other agents. DX-619 was also active against LVX-resistant, gram-positive cocci. The MIC<sub>90</sub> for DX-619 in this group was 2  $\mu\text{g/ml}$ , sixfold lower than that for LVX.

TABLE 2. Antimicrobial activities of DX-619 and other reference compounds against gram-negative anaerobic bacteria and facultative anaerobic bacteria

Organism	MIC ( $\mu\text{g/ml}$ )						
	DX-619	Ceftriaxone	Cefmetazole	Imipenem	Clindamycin	Levofloxacin	Metronidazole
<i>Bacteroides fragilis</i> GAI5562	0.06	8	8	0.5	0.5	1	0.5
<i>Bacteroides fragilis</i> ATCC 25285	0.06	8	8	0.25	1	1	0.5
<i>Bacteroides fragilis</i> NCTC10581	0.06	8	8	0.125	$\leq 0.03$	1	0.5
<i>Bacteroides fragilis</i> GAI0558	0.06	>128	8	0.5	0.5	1	0.5
<i>Bacteroides fragilis</i> GAI7955	0.125	>128	128	8	1	2	0.25
<i>Bacteroides fragilis</i> GAI10150	0.06	>128	64	2	0.5	1	0.5
<i>Bacteroides vulgatus</i> ATCC 8482	0.06	0.06	2	1	0.06	2	0.5
<i>Bacteroides distasonis</i> ATCC 8503	0.06	0.06	2	1	0.06	2	0.5
<i>Bacteroides ovatus</i> ATCC 8483	0.125	128	128	0.25	0.5	4	0.5
<i>Bacteroides thetaiotaomicron</i> ATCC 29741	0.125	128	64	0.5	4	4	0.5
<i>Bacteroides uniformis</i> ATCC 8492	0.125	2	2	0.25	$\leq 0.03$	4	0.5
<i>Bacteroides eggerthii</i> ATCC 27754	0.25	0.5	4	0.125	$\leq 0.03$	4	0.5
<i>Bacteroides ureolyticus</i> NCTC10941	$\leq 0.03$	$\leq 0.03$	0.25	0.25	0.25	0.06	2
<i>Campylobacter gracilis</i> JCM8538	$\leq 0.03$	0.125	8	0.25	0.125	0.06	0.5
<i>Sutterella wadsworthensis</i> ATCC 51579	1	8	8	1	16	0.5	2
<i>Prevotella bivia</i> ATCC 29303	4	32	8	0.06	$\leq 0.03$	8	1
<i>Prevotella buccae</i> ATCC 33574	0.50	0.5	2	0.125	$\leq 0.03$	1	0.5
<i>Prevotella corporis</i> GAI91000	0.50	1	0.5	$\leq 0.03$	$\leq 0.03$	1	0.125
<i>Prevotella heparinolytica</i> ATCC 35895	$\leq 0.03$	0.125	0.5	0.125	$\leq 0.03$	1	0.06
<i>Prevotella intermedia</i> ATCC 25611	0.25	0.125	0.25	0.06	$\leq 0.03$	1	0.5
<i>Prevotella melaninogenica</i> GAI5490	0.25	0.125	0.25	0.06	$\leq 0.03$	1	0.25
<i>Prevotella oralis</i> ATCC 33269	0.5	0.25	2	$\leq 0.03$	$\leq 0.03$	2	0.125
<i>Prevotella oris</i> ATCC 33573	0.125	0.25	1	0.06	$\leq 0.03$	0.5	0.125
<i>Porphyromonas asaccharolytica</i> ATCC 25260	$\leq 0.03$	0.06	0.125	0.06	$\leq 0.03$	0.5	0.125
<i>Porphyromonas gingivalis</i> ATCC 33277	$\leq 0.03$	$\leq 0.03$	0.06	$\leq 0.03$	$\leq 0.03$	0.125	0.03
<i>Fusobacterium nucleatum</i> ATCC 25586	$\leq 0.03$	1	1	0.5	0.06	1	$\leq 0.015$
<i>Fusobacterium varium</i> ATCC 8501	0.5	4	4	4	4	4	0.125
<i>Fusobacterium necrophorum</i> ATCC 25286	$\leq 0.03$	$\leq 0.03$	0.06	0.25	$\leq 0.03$	1	0.125
<i>Bilophilla wadsworthia</i> WAL7959	0.5	2	8	0.5	0.5	0.5	0.06
<i>Desulfobivrio piger</i> DSM749	0.125	4	16	0.25	0.06	0.125	0.25
<i>Capnocytophaga ochracea</i> GAI5586	$\leq 0.03$	$\leq 0.03$	2	0.5	$\leq 0.03$	0.125	2
<i>Veillonella parvula</i> ATCC 10790	0.125	0.5	0.25	0.125	$\leq 0.03$	0.25	1
<i>Veillonella dispar</i> ATCC 17748	0.125	0.25	0.25	0.06	$\leq 0.03$	0.5	2

In the present study, DX-619 showed excellent activity against clinically important anaerobic species. Many antibacterial agents have poor or moderate activity against the *B. fragilis* group. Against the three major species of the *B. fragilis* group (*B. fragilis*, *B. thetaiotaomicron*, and *Bacteroides distasonis*) examined here, DX-619 was the most active agent (MIC<sub>90s</sub> of 0.5, 0.5, and 1  $\mu\text{g/ml}$ , respectively), followed by IPM. As for the anti-*Bacteroides* activities of the other test agents, CMZ was less active against *B. thetaiotaomicron* and *B. distasonis* but active against *B. fragilis*. This is a known tendency of cephamycins (4). CLI still showed strong activity against 68% of *B. fragilis* isolates, with a MIC<sub>50</sub> of 1  $\mu\text{g/ml}$ . The resistance rate of CLI based on the CLSI breakpoint ( $\geq 8 \mu\text{g/ml}$ ) was higher in *B. thetaiotaomicron* (52%) and *B. distasonis* (44%) than in *B. fragilis* (32%). Strains of these species examined in this report were isolated from 2000 to 2002. In 1991, the resistance rates of CLI were 29.0% in *B. fragilis* and 31.7% in *B. thetaiotaomicron*, though a different breakpoint ( $\geq 6.25 \mu\text{g/ml}$ ) and different test media (Gifu anaerobic media) were used in that report (24). This suggests that resistance to CLI is increasing, especially in *Bacteroides* species other than *B. fragilis*. In Japan, the most popular antianaerobic agent, metronidazole, is not available as an antibacterial agent. Under such a situation,

DX-619, which is also active against IPM-resistant *B. fragilis* and *B. thetaiotaomicron*, would be one of the reliable agents.

Besides that, DX-619 showed potent activity against *Prevotella* spp., *Fusobacterium* spp., and anaerobic gram-positive cocci. Species in those groups are frequently isolated from major sites of anaerobic infection, such as the head and neck space and pleuropulmonary site. As well as other infections involving anaerobes, those anaerobic infections are usually polymicrobial infections involving both anaerobic and aerobic bacteria. DX-619 has been reported to have excellent activity against aerobic gram-positive organisms and also strong activity against aerobic gram-negative rods, such as *Escherichia coli* and *Klebsiella pneumoniae* (5). These data indicate that DX-619 has the potential to be used as a single agent in the treatment of polymicrobial infections involving those anaerobic and aerobic species.

Gas gangrene is one of the severe anaerobic infections. The gas gangrene-related species *C. perfringens* (25 strains), *Clostridium novyi* A (4 strains), *Clostridium septicum* (3 strains), and *Clostridium sordellii* (1 strain) were overall sensitive to all agents tested and quite sensitive to DX-619, which inhibited those strains at MICs of  $\leq 0.03$  to 0.25  $\mu\text{g/ml}$ . DX-619 might be useful for this anaerobic infection.

TABLE 3. In vitro activities of DX-619 and other reference compounds against clinical isolates of anaerobic bacteria and facultative anaerobic bacteria

Organism group (no. of isolates)	Antibiotic	MIC ( $\mu\text{g/ml}$ )			% Susceptible at concn ( $\mu\text{g/ml}$ ) of:			
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>	$\leq 2$	$\leq 4$	$\leq 8$	$\leq 16$
<i>Bacteroides distasonis</i> (25)	DX-619	$\leq 0.03$ –4	0.06	1	96.0	100.0	100.0	100.0
	Ceftriaxone	1–>128	128	>128	16.0	20.0	20.0	28.0
	Cefmetazole	16–>128	64	128	0.0	0.0	0.0	8.0
	Imipenem	1–4	1	4	84.0	100.0	100.0	100.0
	Clindamycin	$\leq 0.03$ –>128	4	>128	16.0	56.0	64.0	64.0
	Levofloxacin	0.5–128	1	64	68.0	72.0	72.0	76.0
<i>Bacteroides fragilis</i> (25)	DX-619	0.06–4	0.125	0.5	96.0	100.0	100.0	100.0
	Ceftriaxone	1–>128	8	>128	16.0	24.0	52.0	52.0
	Cefmetazole	8–32	8	16	0.0	0.0	56.0	96.0
	Imipenem	0.125–2	0.5	1	100.0	100.0	100.0	100.0
	Clindamycin	0.125–>128	1	>128	68.0	68.0	68.0	68.0
	Levofloxacin	1–64	2	64	72.0	84.0	88.0	88.0
<i>Bacteroides thetaiotaomicron</i> (25)	DX-619	0.06–1	0.125	0.5	100.0	100.0	100.0	100.0
	Ceftriaxone	64–>128	>128	>128	0.0	0.0	0.0	0.0
	Cefmetazole	64–128	64	128	0.0	0.0	0.0	0.0
	Imipenem	0.25–2	1	1	100.0	100.0	100.0	100.0
	Clindamycin	1–>128	8	>128	24.0	48.0	52.0	52.0
	Levofloxacin	4–64	4	16	0.0	86.0	88.0	92.0
<i>Prevotella</i> spp. (42) <sup>a</sup>	DX-619	0.125–1	0.25	1	100.0	100.0	100.0	100.0
	Ceftriaxone	$\leq 0.03$ –>128	1	32	54.8	57.1	69.0	88.1
	Cefmetazole	0.06–16	0.5	4	83.3	97.6	97.6	100.0
	Imipenem	$\leq 0.03$ –0.25	0.06	0.125	100.0	100.0	100.0	100.0
	Clindamycin	$\leq 0.03$ –>128	$\leq 0.03$	$\leq 0.03$	92.9	92.9	92.9	92.9
	Levofloxacin	0.5–16	0.5	2	97.6	97.6	97.6	100.0
<i>Fusobacterium</i> spp. (25) <sup>b</sup>	DX-619	$\leq 0.03$ –0.5	0.06	0.25	100.0	100.0	100.0	100.0
	Ceftriaxone	$\leq 0.03$ –32	$\leq 0.03$	2	92.0	92.0	96.0	96.0
	Cefmetazole	0.06–16	0.25	8	80.0	88.0	96.0	100.0
	Imipenem	$\leq 0.03$ –4	0.25	4	84.0	100.0	100.0	100.0
	Clindamycin	$\leq 0.03$ –32	0.06	8	88.0	88.0	96.0	96.0
	Levofloxacin	0.5–4	1	2	92.0	100.0	100.0	100.0
<i>Micromonas micros</i> (25)	DX-619	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$	100.0	100.0	100.0	100.0
	Ceftriaxone	0.125–8	0.25	1	92.0	96.0	100.0	100.0
	Cefmetazole	0.25–16	0.5	1	92.0	92.0	96.0	100.0
	Imipenem	0.25–2	0.5	0.5	100.0	100.0	100.0	100.0
	Clindamycin	0.125–16	0.25	0.5	96.0	96.0	96.0	100.0
	Levofloxacin	0.25–0.5	0.5	0.5	100.0	100.0	100.0	100.0
<i>Actinomyces</i> spp. (15) <sup>c</sup>	DX-619	0.06–2	0.125	1	100.0	100.0	100.0	100.0
	Ceftriaxone	$\leq 0.03$ –8	0.25	4	86.7	93.3	100.0	100.0
	Cefmetazole	$\leq 0.03$ –16	0.25	8	80.0	86.7	93.3	100.0
	Imipenem	$\leq 0.03$ –8	1	8	73.3	73.3	100.0	100.0
	Clindamycin	0.125–>128	0.25	1	93.3	93.3	93.3	93.3
	Levofloxacin	0.25–8	8	8	20.0	46.7	100.0	100.0
<i>Propionibacterium acnes</i> (10)	DX-619	0.125	0.125	0.125	100.0	100.0	100.0	100.0
	Ceftriaxone	$\leq 0.03$ –0.125	$\leq 0.03$	0.06	100.0	100.0	100.0	100.0
	Cefmetazole	0.06–0.25	0.125	0.25	100.0	100.0	100.0	100.0
	Imipenem	$\leq 0.03$ –0.06	$\leq 0.03$	0.06	100.0	100.0	100.0	100.0
	Clindamycin	$\leq 0.03$ –0.25	$\leq 0.03$	0.25	100.0	100.0	100.0	100.0
	Levofloxacin	0.25–8	0.5	0.5	90.0	90.0	100.0	100.0
<i>Clostridium difficile</i> (27)	DX-619	0.125–2	0.125	1	100.0	100.0	100.0	100.0
	Ceftriaxone	8–>128	64	>128	0.0	0.0	3.7	7.4
	Cefmetazole	8–>128	32	64	0.0	0.0	4.2	29.2
	Imipenem	2–64	8	16	4.2	16.7	75.0	91.7
	Clindamycin	4–>128	8.0	>128	0.0	20.8	62.5	62.5
	Levofloxacin	4–>128	4	>128	0.0	50.0	54.2	58.3
<i>Clostridium perfringens</i> (25)	DX-619	$\leq 0.03$ –0.25	0.06	0.25	100.0	100.0	100.0	100.0
	Ceftriaxone	$\leq 0.03$ –2	0.125	1	100.0	100.0	100.0	100.0
	Cefmetazole	0.06–0.5	0.125	0.25	100.0	100.0	100.0	100.0
	Imipenem	0.06–0.25	0.125	0.25	100.0	100.0	100.0	100.0
	Clindamycin	$\leq 0.03$ –>128	2	4	84.0	92.0	92.0	92.0
	Levofloxacin	0.25–32	0.5	1	96.0	96.0	96.0	96.0
Other <i>Clostridium</i> spp. (14)	DX-619	$\leq 0.03$ –0.06	$\leq 0.03$	$\leq 0.03$	100.0	100.0	100.0	100.0
	Ceftriaxone	$\leq 0.03$ –8	0.25	8	85.7	85.7	100.0	100.0
	Cefmetazole	$\leq 0.03$ –0.5	0.125	0.25	100.0	100.0	100.0	100.0
	Imipenem	$\leq 0.03$ –0.25	$\leq 0.03$	0.25	100.0	100.0	100.0	100.0
	Clindamycin	$\leq 0.03$ –32	$\leq 0.03$	32	85.7	85.7	85.7	85.7
	Levofloxacin	0.06–1	0.125	0.5	100.0	100.0	100.0	100.0

<sup>a</sup> Includes 28 *P. intermedia/nigrescens* and 10 *P. melaninogenica* isolates and 4 isolates from other *Prevotella* spp.

<sup>b</sup> Includes 10 *F. nucleatum*, 8 *F. necrophorum*, and 5 *F. varium/mortiferum* isolates and 2 isolates from other *Fusobacterium* spp.

<sup>c</sup> One *A. gerencseriae*, two *A. israelii*, three *A. meyeri*, four *A. naeslundii*, and seven *A. odontolyticus* isolates.

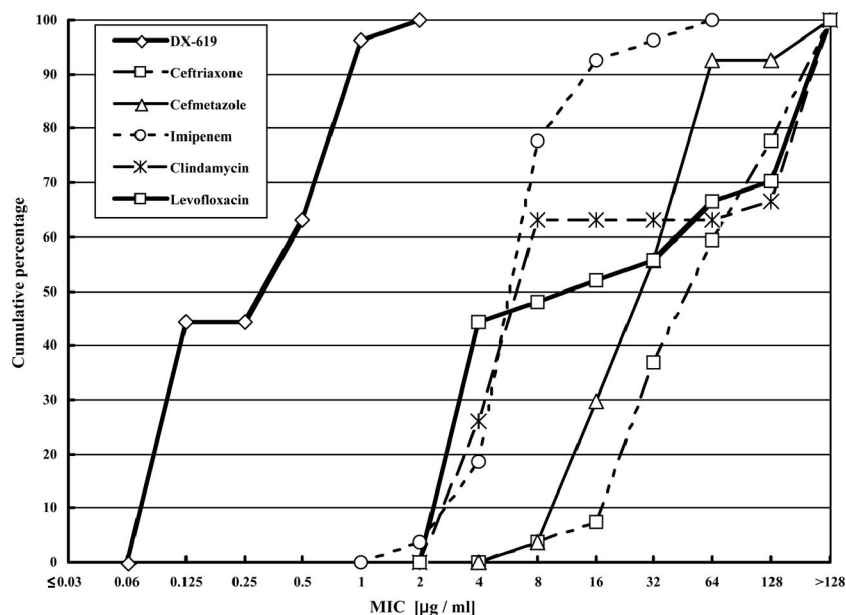


FIG. 1. Antimicrobial activities of DX-619 and other compounds against 27 strains of *C. difficile*.

*C. difficile* is an important nosocomial pathogen that is acquired exogenously, and a variety of clinical outcomes ensue following infection, ranging from asymptomatic colonization to diarrhea to more-severe disease syndromes. It causes disease almost exclusively in the presence of exposure to antimicrobial agents. The most commonly implicated agents were CLI and cephalosporins, but recently, implication of fluoroquinolones was suggested (6, 13, 18). After 2002, outbreaks of a highly lethal type of *C. difficile* were observed in Canada and the United States (13, 14, 19). These strains were reported to be resistant to clinically used newer fluoroquinolones, such as moxifloxacin, gatifloxacin, and LVX (13, 14). Half of the *C. difficile* strains examined in this study were resistant to LVX and CLI, but all strains were susceptible to DX-619 (MIC,  $\leq 2$   $\mu\text{g/ml}$ ). The test strains included four moxifloxacin-resistant strains (the MICs were 8  $\mu\text{g/ml}$  for one strain and 16  $\mu\text{g/ml}$  for the others), and they were also susceptible to DX-619 (MICs of

0.5 to  $\leq 2$   $\mu\text{g/ml}$ ) (data not shown). These observations suggest that DX-619 may be a low-risk agent as an inducer of *C. difficile*-associated diarrhea.

A comparison of our results to the published data on other quinolones shows that, overall, DX-619 covers a wide range of the antianaerobic spectrum, as broad as that for sitafloxacin. Its potent antianaerobic activity seems to be close to that of sitafloxacin and comparable to that of trovafloxacin (1, 3, 8, 11, 15, 20, 21, 25, 26). Fujikawa et al. comprehensively studied the antibacterial activities of DX-619 and reference agents, including IPM and LVX (5). They reported antibacterial activity against *Peptostreptococcus* spp., *C. difficile*, and *B. fragilis* in that paper. Our results were largely consistent with their results. Their results indicated that the antibacterial activities of DX-619 against those anaerobes were more potent than those of garenoxacin, another desfluoroquinolone. During the process of the review of this paper, another study of the antianaerobic

TABLE 4. In vitro activities of DX-619 and other reference compounds against resistant anaerobic strains

Organism group (no. of isolates)	Antibiotic	MIC ( $\mu\text{g/ml}$ )			% Susceptible at concn ( $\mu\text{g/ml}$ ) of:		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>	$\leq 2$	$\leq 4$	$\leq 16$
Imipenem-resistant <i>B. fragilis</i> group organisms (11) <sup>a</sup>	DX-619	0.06–16	0.25	1	90.9	90.9	100.0
	Ceftriaxone	16–>128	128	>128	0.0	0.0	9.1
	Cefmetazole	16–>128	64	128	0.0	0.0	9.1
	Imipenem	8–>128	16	>128	0.0	0.0	36.4
	Clindamycin	1–>128	>128	>128	27.3	36.4	36.4
	Levofloxacin	1–128	4	16	27.3	81.8	90.9
Levofloxacin-resistant anaerobic gram-positive cocci (14) <sup>b</sup>	DX-619	0.125–2	0.25	2	100.0	100.0	100.0
	Ceftriaxone	0.125–16	0.5	8	64.3	78.6	100.0
	Cefmetazole	0.125–2	0.25	2	100.0	100.0	100.0
	Imipenem	$\leq 0.03$ –1	0.06	1	100.0	100.0	100.0
	Clindamycin	$\leq 0.03$ –>128	1	>128	50.0	57.1	64.3
	Levofloxacin	16–128	16	128	0.0	0.0	42.9

<sup>a</sup> Includes 10 *B. fragilis* and 1 *B. thetaiotaomicron* isolate.

<sup>b</sup> Includes five *Finegoldia magna*, eight *Peptoniphilus asaccharolyticus*, and one *Peptostreptococcus anaerobius* isolate.

activity of DX-619 was published in Antimicrobial Agents and Chemotherapy (16). Molitoris et al. compared the activity of DX-619 and those of other antianaerobic agents (amoxicillin-clavulanate, linezolid, meropenem, and moxifloxacin). Though the comparator agents were different from those in this study, we could compare the results of DX-619 treatment for some groups of species tested in both studies (*B. distasonis*, *B. fragilis*, *B. thetaiotaomicron*, *Fusobacterium* spp., *Prevotella* spp., and *Clostridium* spp.). The ranges of MICs, MIC<sub>50</sub>s, and MIC<sub>90</sub>s for DX-619 were almost the same as those for these anaerobes.

In summary, this study showed a potent in vitro activity for DX-619 against clinically important gram-positive and gram-negative anaerobic bacteria. It was also active against LVX- and IPM-resistant strains. Our results indicate that DX-619 would be an effective agent against anaerobic bacteria resistant to other antianaerobic agents and has the potential to be a useful antianaerobic agent for treatment of severe anaerobic infection. To be useful for treatment of community-acquired infections, the novel des-F(6) quinolone DX-619 would have to exhibit higher levels of activity against both gram-positive and gram-negative anaerobic pathogens with various resistance profiles. Clinical studies, including pharmacokinetic/pharmacodynamic studies, will be required to clarify the role of DX-619 in the empirical treatment of community-acquired infections.

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