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In Vitro Evaluation of Faropenem Activity Against Anaerobic Bacteria

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Summary

Faropenem, a new oral penem with broad spectrum activity, could be used as empirical treatment in infections due to unidentified anaerobes, but only a few investigations have been carried out on these bacteria. The aim of this study was to compare faropenem *in vitro* activity with that of positive antimicrobial controls (metronidazole, imipenem, meropenem, amoxicillin, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, cefotetan, cefoxitin and clindamycin) against 462 anaerobic bacterial strains. The reference agar dilution method was used according to the NCCLS standard. Faropenem demonstrated high antimicrobial activity, similar to that of both imipenem and meropenem (faropenem Minimal Inhibitory Concentrations 50% and 90% were 0.12 and 1 mg/L for all Gram-negative anaerobes, 0.25 and 1 mg/L for all Gram-positive anaerobes). Only 5 strains of the *Bacteroides fragilis group* (1.1% of all anaerobes) were resistant to faropenem, which compared favorably with that of other reference antianaerobic drugs. The results obtained confirm those previously reported.

Key words: **Faropenem, activity, anaerobic bacteria, anaerobes, antimicrobial agents.**

INTRODUCTION

Faropenem, a novel broad spectrum β-lactam (penem of the furanem class), is intended for oral administration as a pro-drug ester which makes it of great interest. Moreover, it has proved to be remarkably stable in concentrated solutions, when compared with imipenem and meropenem, in view of potential use by continuous infusion in severe infections [1]. Sharing structural similarities with penicillins and cephalosporins, it has shown antibacterial activity against Gram-positive, Gram-negative bacteria [2,3]. Some authors have reported its good activity especially against pathogens isolated from the respiratory tract [3-8], Enterobacteriaceae and some anaerobes [9-12]. Faropenem is an example of a penem designed to address resistance issues of bacteria responsible for community-acquired infections because of its stability to hydrolysis by beta-lactamases [13], even if an interaction between serum and antibiotic can provoke variations in antibacterial effect that may be species specific. [14] Anaerobic bacteria are often linked to the severity of various infections but it is difficult to isolate and identify anaerobes and the isolates obtained are rarely checked for susceptibility to antibiotics. Faropenem could be useful in empirical oral treatments of infections involving anaerobes. The aim of this study was to contribute to the antianaerobic activity assessment of faropenem. Thus, faropenem Minimal Inhibitory Concentrations (MICs) were compared with those of positive controls (metronidazole, imipenem, meropenem, amoxicillin, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, cefotetan, cefoxitin and clindamycin) against a wide range of Gram-positive and Gram-negative anaerobic bacteria (462 strains).

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MATERIALS AND METHODS

Anaerobes

The 462 strains tested were non-repetitive obligate anaerobic bacteria from the Collection of the Pharmacy Faculty of Lille (CFPL). In fact, they were isolated from human clinical samples over two years and identified according to classical methods, as described in the sixth edition of the Wadsworth-KTL anaerobic bacteriology manual [15]. They were subcultured in Rosenow medium (Bio Rad, Marnes la Coquette, France) and stored in a -20°C freezer when not immediately used. Bacteria purity was checked by Gram staining, subculturing on Columbia blood agar (BioMérieux, Marcy l'Etoile, France) and either laked blood kanamycin-vancomycin plates (Serlabo, France) for *Bacteroides spp*. or josamycin-norfloxacin plates for Fusobacteria [16]. For good quality control and assessment of reproducibility, four reference ATCC control strains were added to each batch of tests when required. The ATCC control strains, advocated by the M11 A3 Norma of the National Committee for Clinical Laboratory Standards [17], were *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, *Clostridium perfringens* ATCC 13124 and *Eggerthella lenta* ATCC 43055. For MIC determination, anaerobic strains were listed as following:

B. fragilis group (199) (*B. fragilis* 85, *B. thetaiotaomicron* 31, *B. ovatus* 19, *B. vulgatus* 27, *B. distasonis* 19, *B. caccae* 4, *B. merdae* 2, *B. uniformis* 8, *B. eggerthii* 1, *B. stercoris* 2, *Bacteroides fragilis* group 1) ; *Prevotella spp* (18) (*P. bivia* 8, *P. buccalis* 1, *P. oulora* 1, *P. intermedia* 6, *P. loescheii* 1, *P. sp* 1); *Porphyromonas spp* (4) (*Po. asaccharolytica* 2, *Po. sp* 2); *Fusobacterium spp* (30) (*F. nucleatum* 25 ,*F. mortiferum* 1 , *F. varium* 1, *F. necrophorum* 2, *F. sp* 1); *Clostridium spp* (77) (*C. perfringens* 29, *C. difficile* 26, *C. baratii* 1, *C. bifermentans* 1, *C. fallax 2, C. histolyticum* 1, *C. ramosum* 3, *C. sphenoides* 2, *C. sporogenes* 2, *C sordelii* 4, *C. septicum* 1, *C. sp 5)*; formerly *Eubacterium spp* (32) (*Egghertella lenta* 21, *Pseudoramibacter alactolyticum* 5, *Colinsella aerofaciens* 1, *E. biforme* 1, *E. ventriosum* 1, *E. saburreum* 1, *E. contortum* 1, *E. sp* 1); *Bifidobacterium spp* (16): *B. sp* 16; *Propionibacterium spp* (11) (*Pr. acnes* 10, *Pr. granulosum* 1); Gram-positive cocci (61) (*Finegoldia magna* 25, *Anaerococcus. prevotii* 10, *Micromonas micros* 10, *Peptostreptococcus anaerobius* 6, *Peptinophilus asaccharolyticus* 4, *Peptostreptococcus morbillorum* 1, *Peptostreptococcus saccharolyticus* 2, *Peptostreptococcus parvulus* 1, *Ruminoccoccus gnavus* 2).

β-lactamase production was checked using the nitrocefin disk method [18].

MIC determination

MICs were determined by a reference agar dilution method according to the Norma M11 T [19] with further recommendations of Norma M11 A2 [20] and M11 A3 [17]. Stock solutions of 512 mg/L of faropenem (Sun 555, Hoechst Marion Roussel, Romainville, France), imipenem (Merck Sharp Dohme, Paris, France), meropenem (Astra-Zeneca, Rueil-Malmaison, France), amoxicillin, ticarcillin (Smith Kline Beecham, Nanterre, France), cefotetan (Astra-Zeneca), cefoxitin (Merck Sharp Dohme, Paris, France), and clindamycin (Pharmacia-Upjohn, Paris, France) were prepared. Combinations with ß-lactamase inhibitors were tested with a fixed 2 µg/mL concentration of clavulanic acid. Metronidazole (Aventis, Paris, France) was first dissolved in 2 mL of methanol and distilled water and then added to the solution. Two-fold dilutions were done in distilled water according to Ericsson and Sherris recommendations [21].

Each antibiotic was incorporated in Wilkins Chalgren agar (Oxoid) to which was added 5% horse sterile defibrinated blood (Eurobio, Les Ulis, France), providing adequate support for the growth of fusobacteria, *Peptostreptococcus sp*, and *Eubacterium spp*. Plates contained serial two-fold dilutions of antimicrobial agents (ranging from 512 mg/L to 0.03 mg/L of clindamycin, from 256 mg/L to 0.06 mg/L of ticarcillin combined with clavulanic acid and cefoxitin, from 256 mg/L to 0.03 mg/L of metronidazole, from 256 mg/L to 0.0015 mg/L of faropenem, imipenem, meropenem and cefotetan, from 128 mg/L to 0.003 mg/L of amoxicillin and amoxicillin combined with clavulanic acid). Amoxicillin and ticarcillin combined with clavulanic acid were diluted to obtain the constant concentration of 2 µg/mL of the β–lactamase inhibitor as is usual in most European countries. All plates were used within 24 hours of preparation.

An active growing culture in Rosenow medium was diluted in Schaedler broth (BioMérieux) to reach and match the 0.5 point of a MacFarland standard. Hemin (5 µg/L), menadione (0.1 µg/L), sodium bicarbonate (1 g/L) and 1% (v/v) laked blood were added to the Schaedler broth for fastidious strains. A Mast multipoint inoculator (Mast Systems, London, U.K.) was used to deliver inocula of approximately 10^5 CFU per spot on the agar plates. Plates were incubated in an anaerobic chamber (Forma Scientific, Marietta, OH, USA) at 35° to 36°C. At the end of each series of tests, two plates of Wilkins Chalgren agar were also inoculated without antimicrobial agent. They were anaerobically and aerobically incubated either to serve as positive control for organism viability, or to indicate possible aerobic contamination. The MIC reading was done after 48h of incubation. The categorization of the MIC values in clinical categories was done according to the NCCLS breakpoints. For meropenem and faropenem, breakpoints were equivalent to those of imipenem.

RESULTS

MIC_{50} and MIC_{90} values for each antibiotic and for each group of bacteria are listed in *Table 1*. Percentages of susceptibility and resistance at the NCCLS breakpoints are listed in *Tables 2 and 3*.

Gram-negative anaerobes

Bacteroides: For the *B. fragilis* group, the 199 strains tested were susceptible (98.5%) to faropenem and other carbapenems. MIC_{50} on this group of anaerobes was 0.12 mg/L for faropenem, com-

TABLE 1- *In vitro comparative activity of faropenem and nine reference drugs tested against 462 clinical isolates of anaerobic bacteria.*

Microorganisms and antimicrobial agents	MIC (mg/L) 50%	90%	Range
Bacteroides fragilis (85)			
Metronidazole	0.25	2	0.03-8
Faropenem	0.01	0.5	0.015->128
Imipenem	0.03	0.5	0.015-128
Meropenem	0.12	0.5	0.03->128
Amoxicillin	16	>64	0.25->64
Amoxicillin-clavulanic acid	0.25	4	0.06->64
Ticarcillin-clavulanic acid	0.12	2	0.06->128
Cefotetan	4	32	2->128
Cefoxitin	8	16	2-128
Clindamycin	0.25	256	0.03->256
Bacteroides thetaiotaomicron (31)			
Metronidazole	0.5	1	0.12-4
Faropenem	0.12	0.5	0.015-4
Imipenem	0.12	0.25	0.015-2
Meropenem	0.25	0.25	0.12-4
Amoxicillin	32	>64	16->64
Amoxicillin-clavulanic acid	0.25	8	0.25->64
Ticarcillin-clavulanic acid	0.5	4	0.25->128
Cefotetan	32	64	16->128
Cefoxitin	16	32	8->128
Clindamycin	0.25	256	0.03->256
Bacteroides vulgatus (27)			
Metronidazole	0.25	0.5	0.03-4
Faropenem	0.25	2	0.015-2
Imipenem	0.125	0.25	0.015-1
Meropenem	0.25	0.5	0.06-1
Amoxicillin	>64	>64	0.5->64
Amoxicillin-clavulanic acid	0.25	16	0.06->64
Ticarcillin-clavulanic acid	0.25	16	0.06-64
Cefotetan	32	>128	1->128
Cefoxitin	8	32	1-64
Clindamycin	0.12	256	0.03-256
Bacteroides of the *fragilis* group (199) [a]			
Metronidazole	0.5	1	0.03-8
Faropenem	0.12	1	0.015->128
Imipenem	0.06	0.5	0.015->128
Meropenem	0.25	1	0.03->128
Amoxicillin	32	>64	0.25->64
Amoxicillin-clavulanic acid	0.25	8	0.06->64
Ticarcillin-clavulanic acid	0.25	8	0.06->128
Cefotetan	16	128	1->128
Cefoxitin	8	32	1->128
Clindamycin	0.25	256	0.03->256

TABLE 1- *Continued*

Microorganisms and antimicrobial agents	MIC (mg/L) 50%	90%	Range
Prevotella spp (18) [b]			
Metronidazole			0.03-1
Faropenem			0.015-0.12
Imipenem			0.015-0.12
Meropenem			0.015-1
Amoxicillin			0.06->64
Amoxicillin-clavulanic acid			0.03-8
Ticarcillin-clavulanic acid			0.06-16
Cefotetan			0.015-8
Cefoxitin			0.06-4
Clindamycin			≤0.03
Porphyromonas spp (4) [c]			
Metronidazole			0.03-0.25
Faropenem			0.03-1
Imipenem			0.015-0.25
Meropenem			0.03-0.12
Amoxicillin			0.03-1
Amoxicillin-clavulanic acid			0.03-1
Ticarcillin-clavulanic acid			0.25-16
Cefotetan			0.06-4
Cefoxitin			0.25-16
Clindamycin			0.03-0.5
Fusobacterium spp (30) [d]			
Metronidazole	0.03	0.25	0.03-0.5
Faropenem	≤0.015	0.06	0.015-1
Imipenem	0.03	0.25	0.015-0.5
Meropenem	≤0.015	≤0.015	0.015-0.06
Amoxicillin	0.03	1	0.03-8
Amoxicillin-clavulanic acid	0.03	0.06	0.03-2
Ticarcillin-clavulanic acid	0.06	1	0.06-4
Cefotetan	0.01	2	0.015-32
Cefoxitin	0.12	1	0.06-4
Clindamycin	0.03	0.06	≤0.03-8
Veillonella spp. (11)			
Metronidazole			0.12-1
Faropenem			0.06-2
Imipenem			0.015-1
Meropenem			0.015-0.12
Amoxicillin			0.03-2
Amoxicillin-clavulanic acid			0.03-4
Ticarcillin-clavulanic acid			0.06-64
Cefotetan			0.06-8
Cefoxitin			0.06-4
Clindamycin			0.03-0.12
All Gram-negative anaerobes (265)			
Metronidazole	0.25	1	0.03-8
Faropenem	0.12	1	0.015->128
Imipenem	0.06	0.5	0.015->128
Meropenem	0.12	0.5	0.015->128
Amoxicillin	16	>64	0.03->64
Amoxicillin-clavulanic acid	0.25	8	0.03->64
Ticarcillin-clavulanic acid	0.25	8	0.06->128
Cefotetan	8	64	0.015->128
Cefoxitin	8	32	0.06->128
Clindamycin	0.25	256	0.03->256

TABLE 1- *Continued*

Microorganisms and antimicrobial agents	MIC (mg/L) 50%	90%	Range
Clostridium perfringens (29)			
Metronidazole	0.25	0.5	0.03-1
Faropenem	0.25	0.5	0.03-0.5
Imipenem	0.06	0.06	0.015-0.12
Meropenem	≤0.015	≤0.015	0.015-0.06
Amoxicillin	0.03	0.06	0.03-0.12
Amoxicillin-clavulanic acid	0.03	0.03	0.03-0.12
Ticarcillin-clavulanic acid	0.25	0.5	0.06-1
Cefotetan	0.06	4	0.015-2
Cefoxitin	0.5	1	0.12-2
Clindamycin	0.03	1	0.03-2
Clostridium difficile (26)			
Metronidazole	0.25	0.5	0.03-1
Faropenem	1	2	0.03-2
Imipenem	2	2	0.015-2
Meropenem	1	2	0.03-2
Amoxicillin	0.5	2	0.12-4
Amoxicillin-clavulanic acid	0.5	2	0.06-2
Ticarcillin-clavulanic acid	8	16	0.25-32
Cefotetan	8	16	0.12-16
Cefoxitin	64	64	32-64
Clindamycin	1	16	0.12-128
Other *Clostridium* (22) [e]			
Metronidazole	0.25	0.5	0.03-0.5
Faropenem	0.12	2	0.015-2
Imipenem	0.06	1	0.03-1
Meropenem	0.06	1	0.015-1
Amoxicillin	0.12	0.5	0.03-1
Amoxicillin-clavulanic acid	0.25	0.5	0.03-2
Ticarcillin-clavulanic acid	0.5	16	0.25-32
Cefotetan	0.25	>128	0.015->128
Cefoxitin	1	64	0.06-64
Clindamycin	1	2	0.03-16
formerly *Eubacterium spp.* (32) [f]			
Metronidazole	0.25	1	0.12-2
Faropenem	1	2	0.015-2
Imipenem	0.25	0.5	0.015-1
Meropenem	0.25	0.5	0.015-0.5
Amoxicillin	0.5	1	0.03-8
Amoxicillin-clavulanic acid	0.5	1	0.03-2
Ticarcillin-clavulanic acid	0.5	4	0.06-64
Cefotetan	32	64	0.015->128
Cefoxitin	8	16	0.12-32
Clindamycin	0.25	2	0.015-128
Propionibacterium spp. (11) [g]			
Metronidazole			32->128
Faropenem			0.015-0.12
Imipenem			<0.015
Meropenem			0.015-0.12
Amoxicillin			0.03-0.12
Amoxicillin-clavulanic acid			0.03-0.25
Ticarcillin-clavulanic acid			0.06-1
Cefotetan			0.03-1
Cefoxitin			0.06-1
Clindamycin			≤0.03

TABLE 1- *Continued*

Microorganisms and antimicrobial agents	MIC (mg/L) 50%	90%	Range
Anaerobic Gram-positive cocci (61) [h]			
Metronidazole	0.25	1	0.03-64
Faropenem	0.12	0.5	0.015-1
Imipenem	0.03	0.25	0.015-2
Meropenem	0.06	0.25	0.015-0.5
Amoxicillin	0.12	0.5	0.03-8
Amoxicillin-clavulanic acid	0.06	0.25	0.03-1
Ticarcillin-clavulanic acid	0.5	8	0.06-32
Cefotetan	0.5	4	0.015-32
Cefoxitin	0.5	2	0.06-4
Clindamycin	0.12	1	0.03-128
All Gram-positive anaerobes (197)			
Metronidazole	0.25	1	0.03->128
Faropenem	0.25	1	0.015-2
Imipenem	0.06	1	0.015-2
Meropenem	0.12	0.5	0.015-2
Amoxicillin	0.12	1	0.03-8
Amoxicillin-clavulanic acid	0.12	1	0.03-2
Ticarcillin-clavulanic acid	1	16	0.06-64
Cefotetan	1	32	0.015->128
Cefoxitin	1	64	0.06-64
Clindamycin	0.12	2	0.03-128

[a] Comprises: *B. fragilis* 85, *B. thetaiotaomicron* 31, *B. vulgatus* 27, *B. distasonis* 19, *B. ovatus* 19, *B. caccae* 4, *B. merdae* 2, *B. uniformis* 8, *B. eggerthii* 1, *B. stercoris* 2, *Bacteroides fragilis* group 1.
[b] Comprises: *P. bivia* 8, *P. buccalis* 1, *P. oulora* 1, *P. intermedia* 6, *P. loescheii* 1, *P. sp* 1.
[c] Comprises: *Po. asaccharolytica* 2, *Po. sp* 2.
[d] Comprises: *F. nucleatum* 25 ,*F. mortiferum* 1 , *F. varium* 1, *F. necrophorum* 2, *F. sp* 1.
[e] Comprises: *C. baratii* 1, *C. bifermentans* 1, *C. fallax 2, C. histolyticum* 1, *C. ramosum* 3, *C. sphenoides* 2, *C. sporogenes* 2, *C. sordelii* 4, *C. septicum* 1, *C. sp 5.*
[f] Comprises: *Egghertella lenta* 21, *Pseudoramibacter alactolyticum* 5, *Colinsella aerofaciens* 1, *Eubacterium biforme* 1, *Eubacterium ventriosum* 1, *Eubacterium saburreum* 1, *Eubacterium contortum* 1, *Eubacterium. sp* 1.
[g] Comprises: *Pr. acnes* 10, *Pr. granulosum* 1.
[h] Comprises: *Finegoldia magna* 25, *Anaerococcus. prevotii* 10, *Micromonas micros* 10, *Peptostreptococcus anaerobius* 6, *Peptinophilus asaccharolyticus* 4, *Peptostreptococcus morbillorum* 1, *Peptostreptococcus saccharolyticus* 2, *Peptostreptococcus parvulus* 1, *Ruminoccoccus gnavus* 2.

TABLE 2 - *Comparative antimicrobial activities against anaerobes (% of susceptibility).*

Microorganisms (N°)	MTR (≤8)	FAR[a] (≤4)	IMI (≤4)	MER[a] (≤4)	AMC[b] (≤4/2)	TCC[b] (≤32/2)	CTT (<16)	CFX (≤16)	CLN (≤2)
B. fragilis group (199)	100	97	98.5	98.5	85.4	96.9	56.8	85.4	72.9
other Gram-negative anaerobes (66)	100	100	100	100	98.5	98.5	95.4	98.4	97
C. perfringens (29)	100	100	100	100	100	100	100	100	100
C. difficile (26)	100	100	100	100	100	100	92.3	0	69.2
other clostridia (22)	100	100	100	100	100	100	72.7	68.1	90.9
other Gram (+) rods (59)	74.6	100	100	100	100	96.6	67.8	96.6	93.2
Gram (+) cocci (61)	95.1	100	100	100	100	100	98.3	100	90.1
all anaerobes (462)	96.1	98.7	99.3	99.3	93.2	98.9	74.7	85.9	83.5

[a] The NCCLS breakpoint is not yet established for anaerobes and faropenem. Therefore, the imipenem value was chosen.
[b] Amoxicillin + clavulanic acid and ticarcillin + clavulanic acid were tested with a constant 2 µg/mL concentration of clavulanic acid.
There is no NCCLS breakpoint for Gram-positive anaerobes.
MTR = metronidazole, FAR = faropenem, IMI = imipenen, MER = meropenem, AMX = amoxicillin, AMC = amoxicilin + clavulanic acid, TCC = ticarcillin+ clavulanic acid, CTT = cefotetan, CFX = cefoxitin, CLN = clindamycin.

pared to that of imipenem (0.06 mg/L), meropenem (0.25 mg/L), metronidazole and amoxicillin-clavulanic acid (0.5 mg/L). The resistance rate for faropenem (2.5%) calculated at the 16 mg/L breakpoint, was similar to that of anti-anaerobic drugs such as imipenem (1.5%), meropenem (1.5%) and ticarcillin-clavulanic acid (2.5%). Resistance to faropenem was not as frequent as resistance to cefotetan (27.1%), cefoxitin (6%) and clindamycin (20.6%). No resistance to metronidazole was detected. Only 5 strains were resistant to faropenem (MIC≥16 mg/L). One strain was in the intermediate clinical category (MIC 8 mg/L). Faropenem MICs were ≥ 4 mg/L for nine strains that were further classified into 3 groups according to their antibiotic susceptibility profile (*Table 4*). Group I includes 3 *B. fragilis* strains (9328, 9329 and 9330) that were resistant to all ß-lactams including faropenem (MIC >128 mg/L). These strains also had decreased susceptibility (MIC 2 or 4 mg/L) to metronidazole and to clindamycin (MIC 2 mg/L). In group II, *B. thetaiotaomicron* 9302 was resistant to amoxicillin-clavulanic, ticarcillin-clavulanic acid, cefoxitin and cefotetan but remained susceptible to imipenem and faropenem (MIC ≤4 mg/L). Group III includes 4 *B. ovatus* strains (95214, 95172, 95214, 92218) and

TABLE 3 - *Comparative activities of tested antibiotics against anaerobes (% of resistance).*

Microorganisms (N°)	**MTR (≥32)**	**FAR[a] (≥16)**	**IMI (≥16)**	**MER[a] (≥16)**	**AMC[b] (≥16/2)**	**TCC[b] (≥128/2)**	**CTT (≥64)**	**CFX (≥64)**	**CLN (≥8)**
B. fragilis group (199)	0	2.5	1.5	1.5	8.0	2.5	27.1	6	20.6
other Gram-negative anaerobes (66)	0	0	0	0	0	0	1.5	0	1.5
C. perfringens (29)	0	0	0	0	0	0	0	0	0
C. difficile (26)	0	0	0	0	0	0	0.7	84.6	26.9
other clostridia (22)	0	0	0	0	0	0	27.3	18.1	0
other Gram (+) rods (59)	25.4	0	0	0	0	0	21.9	0	4.5
Gram (+) cocci (61)	4.9	0	0	0	0	0	0	0	9.8
all anaerobes (462)	3.9	1.1	0.6	0.6	3.7	1.1	15.1	8.2	12.8

Same legend as in table 2

TABLE 4 - *Antibiotic susceptibility profile for the strains that showed ≥ 4 mg/L faropenem MICs.*

Strains	**Antibiotics and MICs in mg/L**							
	AMC[b]	**TCC[b]**	**CFX**	**CTT**	**FAR[a]**	**MER[a]**	**IMI**	**MTR**
Group I								
B. fragilis 9328	>64	>128	64	256	>64	>64	16	2
B. fragilis 9329	>64	>128	128	>256	>64	>64	>64	4
B. fragilis 9330	>64	>128	64	256	>64	>64	>64	2
Group II								
B. thetaiotaomicron 9302	32	>128	256	>256	4	4	2	0.5
Group III								
B. ovatus 92128	4	4	64	256	8	2	1	0.5
B. ovatus 95172	8	8	64	512	4	1	1	1
B. ovatus 95214 a	8	8	128	>512	16	2	0.25	0.25
B. ovatus 95214 b	4	4	128	>512	16	2	0.5	0.25
B. fragilis 92215	4	0.25	16	64	4	2	0.5	8

[a] The NCCLS breakpoint is not yet established for anaerobes and faropenem. Therefore, the imipenem value was chosen.

[b] Amoxicillin + clavulanic acid and ticarcillin+ clavulanic acid were tested with a constant 2 µg/mL concentration of clavulanic acid.

MTR = metronidazole, FAR = faropenem, IMI = imipenen, MER = meropenem, AMC = amoxicilin + clavulanic acid, TCC = ticarcillin+ clavulanic acid, CTT = cefotetan, CFX = cefoxitin.

one *B. fragilis* strain 92215. These 5 strains were resistant to both cefoxitin and cefotetan but MICs for amoxicillin-clavulanic acid were in the 4-8 mg/L range and faropenem was less efficient than imipenem.

Resistance to amoxicillin-clavulanic acid was proved for 8 other strains that were susceptible to ticarcillin-clavulanic acid, faropenem and carbapenems. Among the 41 clindamycin-resistant strains, 24 strains showed no other antibiotic resistance whereas 13, 6, and 4 strains were also resistant to cefotetan, amoxicillin-clavulanic acid or both cefotetan and amoxicillin-clavulanic acid, respectively. All cefoxitin-resistant strains were resistant to cefotetan. Resistance to metronidazole was not found, but 6 strains had decreased susceptibility to metronidazole (MIC from 4 to 8 mg/L).

Other Gram-negative anaerobes

For the other Gram-negative anaerobes, amoxicillin MICs ≥1 mg/L were associated with a positive nitrocefin test for *Prevotella* and *Fusobacterium* (61% and 10% of the strains, respectively). This was never observed among *Porphyromonas* strains. All those strains were susceptible to the other antimicrobial agents except for one *F. necrophorum* strain that was resistant to clindamycin. All *Fusobacterium, Prevotella* and *Porphyromonas* strains were inhibited by 1 mg/L of either faropenem, imipenem, meropenem or metronidazole whereas higher concentrations were required to stop their growth with the other antibiotics.

Gram-positive anaerobes

All Gram-positive anaerobes were susceptible to faropenem. The growth of all strains was indeed inhibited at a concentration of 2 mg/L.

Sporulated Gram-positive bacilli: Among the sporulated Gram-positive anaerobic bacilli, *C. perfringens* was susceptible to all the antibiotics tested; a concentration of 0.5 mg/L for faropenem was sufficient to inhibit all the strains investigated. For faropenem and meropenem, 1 mg/L MIC was found with most of the *C. difficile* strains tested. For the clostridia other than *C. difficile* and *C. perfringens*, resistance to cefotetan, cefoxitin or clindamycin occurred for 27.3, 18.1 and 9.1% of the strains, respectively. Finely, 2 mg/L of faropenem was able to inhibit all clostridia.

Non-sporulated Gram-positive bacilli: According to their susceptibility to metronidazole, the non-sporulated Gram-positive bacilli are divided into two groups. Formerly *Eubacterium* gender and two-thirds of *Bifidobacterium* strains were susceptible to metronidazole (MIC ≤4 mg/L). *Propionibacterium spp.* were resistant to this antibiotic (MIC ≥32 mg/L). Some *Eubacterium spp* strains were also resistant to cefotetan. Faropenem was very effective against *Bifidobacterium spp.* (8 of the 16 strains isolated were inhibited by 0.25 mg/L), *Propionibacterium spp.* (6 of the 11 strains tested were inhibited by 0.03 mg/L), and *Eubacterium* (MIC_{50} of 1 mg/L). Other antibiotics that do not belong to the 5-nitroimidazoles were very effective against these three anaerobic genera.

Gram-positive cocci

All ß-lactams were very effective against the Gram-positive coccal strains and 1 mg/L of faropenem inhibited all the investigated strains

The results of the tested faropenem activity compared with that of two carbapenems against the 462 anaerobes proved to be similar for the 3 antimicrobial agents.

DISCUSSION

As anaerobic bacterial susceptibility to antimicrobial agents varies according to the genus, species and strains themselves, testing a broad range of anaerobes seemed essential to evaluate faropenem as a potential agent for appropriate empiric therapy.

Faropenem was highly active against the strains of the *B. fragilis* group, similarly to imipenem and meropenem. This was shown by Goldstein *et al.* on three strains of *B. fragilis* isolated from skin and soft tissue infections from animal and human bites [10] and by studies of time-kill kinetics by Boswell on 3 strains of *B. fragilis* [22] even though the number of strains they studied was smaller than ours. The *B. fragilis* group often causes infections below the diaphragm and over the last decade, the opportunistic pathogens of this bacteria group have proved especially resistant to antimicrobial agents [23-25]. Most of this group of strains produce a chromosomic β-lactamase that hydrolyses antibiotics that are often used for a initial therapy [26,27]. The resistance rates (*Table 3*) calculated for faropenem (2.5%), similar to that of the other penems tested and ticarcillin-clavulanic acid, are lower than those of amoxicillin-clavulanic acid (8%) and other anti-anaerobic drugs widely used in prophylaxis for colorectal surgery. In the *B. fragilis* group, resistance to all ß-lactams including carbapenems is due to the production of a carbapenemase meanwhile mechanisms of cross-resistance to ß-lactams other than carbapenems are: hyperproduction of the chromosomic cephalosporinase *cepA* [26,27], decreased permeability of the bacteria barrier by lack of porins [28-29], or multi-drug efflux pump [30], production of a silent carbapenemase [31-33], alteration of penicillin binding proteins [34] or a combination of these mechanisms. Further investigations (using polymerase chain reaction) were carried out (results not shown here) that showed why faropenem MICs could reach values ≥4 mg/L (*Table 4*): Group I includes 3 *B. fragilis* strains producing a carbapenemase (due to *cfiA* gene and upstream insertion sequence element) [31-33], thus, they were

resistant to all ß-lactams including faropenem. This is associated with decreased susceptibilty to metronidazole and clindamycin. The susceptibility pattern of *B. thetaiotaomicron* of group II - resistant to amoxicillin-clavulanic, ticarcillin-clavulanic acid, cefoxitin and cefotetan, but still remaining susceptible to imipenem and faropenem - could be explained by a silent carbapenemase [35]. As for the four *B. ovatus* strains and the *B. fragilis* strain of the Group III, that are less susceptible to faropenem than to imipenem, their new resistance profile allowed us to suppose that altered Penicillin Binding Protein (PBP) are involved but the mechanism of this particular resistance is not proved yet. Similar interesting results were previously described for *B. fragilis* that was less susceptible to faropenem (intermediate MICs) than to imipenem [11]. The lack of porin [28,36] and/or the hyperproduction of the chromosomal cephalosporinase are the main causes of resistance to amoxicillin-clavulanic acid observed for the 8 strains susceptible to ticarcillin-clavulanic acid, faropenem and carbapenems.

Faropenem proved to be one of the more potent agent against Gram-negative anaerobes other than the *B. fragilis* group. *Fusobacterium*, *Prevotella* and *Porphyromonas* were inhibited by 1 mg/L of faropenem and other penems. These species - frequently involved in ear, nose and throat and lower respiratory tract infections - were also studied by Goldstein *et al*. [10] who tested more specifically anaerobes from infections due to bites and who found similar results. Wexler *et al*. [11] reported growth inhibition at 2 or 4 mg/L of faropenem for some strains of the *Fusobacterium mortiferum/varium* group, for *Porphyromonas levii*-like organisms or for *Prevotella*; however, faropenem had good activity against those Gram-negative bacteria.

Among the sporulated Gram-positive bacilli tested, all clostridia were inhibited by 2 mg/L or lower concentrations of faropenem whereas intrinsic resistance to cefoxitin among *C. difficile* is well known and resistance to cefotetan and/or clindamycin was also found as shown in *Table 3*. MICs reported by Wexler *et al*. [11] for faropenem and *C. difficile* were higher with 2/11 strains (16 and 32 µg/mL) that were also resistant to imipenem.

The non-sporulated Gram-positive bacilli are generally divided into two groups that are obviously represented in our study: on the one hand, the former *Eubacterium spp.* group and 2/3 of the *Bifidobacterium* strains that were susceptible to metronidazole and on the other hand, 1/3 of the *Bifidobacterium* strains and the *Propionibacterium* spp that are intrinsically resistant to this antibiotic (5-nitroimidazole). In all cases, faropenem showed high activity against these bacteria as previously described by Wexler *et al*. [11] and Goldstein *et al*. [10].

Gram-positive cocci are usually known to be susceptible to metronidazole and clindamycin in France, except for about 10% or less [24,25,37,38]. In agreement with data from Woodcock *et al*. [3] and Goldstein *et al*. [10], our strains were inhibited at concentrations of faropenem ≤1 mg/L.

The breakpoints for faropenem are not yet established. As this penem is intended to be orally administered, other breakpoints could emerge in the future with low incidence on the antibacterial activity against anaerobes.

Faropenem was tested against a large number of clinical isolates of Gram-positive and Gram-negative anaerobic bacteria. Metronidazole, amoxicillin, amoxicillin-clavulanic acid, ticarcillin- clavulanic acid, cefotetan, cefoxitin, imipenem, meropenem and clindamycin were used as positive controls. Faropenem showed high activity against anaerobes, similar to that of imipenem and meropenem. Only 5 strains (1.1% of the 462 anaerobes) were resistant to faropenem including three *Bacteroides fragilis* that produced a carbapenemase and two *Bacteroides ovatus* strains. This resistance rate was similar to that of other reference anti-anaerobic drugs.

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