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In Vitro Activities of Doripenem and Comparator Agents against 364 Anaerobic Clinical Isolates

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The in vitro activities of doripenem against 364 anaerobic isolates were measured and compared to those of ertapenem, imipenem, meropenem, ceftriaxone, and levofloxacin. All of the carbapenems were active against nearly all *Bacteroides fragilis* group isolates. Doripenem was either comparable to or slightly less active than imipenem and meropenem against most isolates but more active than the other penems against *Clostridium difficile*. Doripenem appears to have excellent activity against a broad range of anaerobes.

Doripenem, a 1-β-methyl carbapenem being developed for the treatment of serious systemic bacterial infections, is resistant to hydrolysis by dihydropeptidase 1 (7). In aerobes, doripenem appears to have the advantages of both imipenem (in its activity against gram-positive cocci) and meropenem (in its activity against gram-negative organisms) (12). Metalloenzymes that hydrolyze carbapenems have been found in both aerobic bacteria (3, 10, 11) and anaerobic bacteria (2); the gene for the metalloenzyme may be silent or expressed to various degrees, resulting in a wide range of carbapenem resistance levels (13). In Japan, this accounts for the 2 to 4% rate of resistance to imipenem (1, 16), but these isolates are rarely found in the United States. The purpose of this study was to measure the efficacy of doripenem against a wide range of clinical anaerobic isolates and to compare its in vitro activities to those of other antimicrobial agents.

Bacteria were clinical isolates collected from a wide range of infections throughout the United States or worldwide and identified at the Wadsworth Anaerobe Laboratory (5). MICs were determined by the CLSI (formerly NCCLS)-approved Wadsworth agar dilution technique (8). Antimicrobial agents were obtained from the following companies: doripenem (Shionogi & Co., Ltd., Osaka, Japan), imipenem and ertapenem (Merck, Rahway, NJ), meropenem (AstraZeneca, Waltham, MA), ceftriaxone (Hoffman La Roche, Nutley, NJ), and levofloxacin (Johnson and Johnson, Raritan, NJ). For analysis purposes, the bacteria tested were placed in species or genus groups with >5 isolates, and the MIC ranges, mean geometric MICs, MIC_{50}s, and MIC_{90}s were reported. Susceptible (intermediate) breakpoints are indicated in Table 1.

Efflux inhibitor studies were performed by the spiral gradient endpoint system (19, 20) by first depositing a uniform concentration of efflux inhibitor on 15 mm brucella blood agar plates (Anaerobe Systems), resulting in the following concentrations: carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Sigma), 12.5 μg/ml; CCCP, 25 μg/ml; MC 207,110 (Sigma), 100 μg/ml, reserpine (Sigma), 25 μg/ml; and verapamil (Sigma), 100 μg/ml. Doripenem was then deposited in the gradient mode, and MICs were determined. The concentrations of efflux inhibitors chosen were not inhibitory for the strains.

Doripenem was active against almost all strains of the *Bacteroides fragilis* group of organisms. Doripenem had MICs of 16 μg/ml for one strain and MICs of 8 μg/ml for three additional strains. The MICs of the other carbapenems for these strains were one to twofold dilutions lower but also were elevated compared to their MICs for other *B. fragilis* group strains. The mode for doripenem was quite clear at 0.5 μg/ml. While the mode for meropenem was twofold dilutions lower and the modes for imipenem and ertapenem were 0.25 and 0.5 μg/ml, respectively, the distribution of strains around the respective modes was wider than that for doripenem. No significant difference was detected in doripenem MICs for strains collected before 2000 or in the years 2000 to 2003 (geometric mean MICs were 0.54, 0.57, 0.5, 0.4, and 0.29 μg/ml, respectively).

The presence of efflux pump inhibitors CCCP, MC 207,110, reserpine, and verapamil did not affect the geometric mean MIC when all strains were considered together (the range was 0.30 μg/ml to 0.38 μg/ml). For strains with higher MICs (>1 μg/ml), a decrease in the geometric mean MIC was observed with the inhibitors (1.85 μg/ml versus 0.89 to 1.1 μg/ml). When strains with known metalloenzymes (which result in very high MICs of carbapenems) were deleted from the analysis, the geometric mean MICs were ~2-fold lower in the presence of the efflux inhibitors (1.57 μg/ml versus 0.68 to 0.81 μg/ml). No difference was seen in doripenem MICs with or without inhibitors for the two strains with metalloenzymes in the concentration range used in these studies. Since these MICs are clearly within the susceptible range, the efflux of these agents is not a clinically significant resistance mechanism. However, these data do indicate that these pumps are capable of pumping out doripenem and that overexpression of efflux pumps might contribute to the development of resistance.

Doripenem, meropenem, and ertapenem had slightly elevated MICs for one strain of ceftriaxone-resistant *Porphyromonas gingivalis*. All other *Porphyromonas* strains were inhibited by ≤4 μg/ml of the agents. *Prevotella bivia*, a major gynecological pathogen (6), was susceptible to all of the agents tested at

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TABLE 1. Activities of doripenem and comparator agents[a]

Organism (n[b]) and antimicrobial agent	MIC_{50}	MIC_{90}	Geometric mean MIC	Range	%S	%I	%R
B. fragilis (81)							
Doripenem (116)[c]	0.5	1	0.5	0.25–16	96	3	1
Ertapenem	0.25	0.5	0.3	0.12–4	100	0	0
Imipenem	0.25	0.5	0.2	0.06–2	100	0	0
Meropenem	0.12	0.5	0.2	0.12–8	97	3	0
Ceftriaxone	32	>32	25.7	8–>32	15	48	37
Levofloxacin	2	2	1.5	0.50–8	91	5	4
B. ovatus (20)							
Doripenem (27)	0.5	1	0.4	0.12–2	100	0	0
Ertapenem	0.5	1	0.5	0.12–2	100	0	0
Imipenem	0.25	0.5	0.2	0.06–0.50	100	0	0
Meropenem	0.25	0.5	0.2	0.03–0.50	100	0	0
Ceftriaxone	32	>32	25	8–>32	20	50	30
Levofloxacin	8	16	7.5	4–16	0	25	75
B. thetaiotaomicron (42)							
Doripenem (44)	0.5	1	0.6	0.12–2	100	0	0
Ertapenem	0.5	2	0.6	0.06–2	100	0	0
Imipenem	0.5	1	0.4	0.03–1	100	0	0
Meropenem	0.25	0.5	0.3	0.06–1	100	0	0
Ceftriaxone	32	>32	31.1	16–>32	2	58	40
Levofloxacin	8	8	6.3	1–>32	2	29	69
Other *B. fragilis* grp. species (23)							
Doripenem (39)	0.5	2	0.6	0.12–16	98	0	2
Ertapenem	0.5	2	0.6	0.12–2	100	0	0
Imipenem	0.5	1	0.4	0.03–1	100	0	0
Meropenem	0.25	0.5	0.3	0.06–1	100	0	0
Ceftriaxone	32	>32	30.3	16–>32	4	53	43
Levofloxacin	2	16	3.9	1–32	52	13	35
Bilophila wadsworthia (21)							
Doripenem	0.12	0.12	0.1	0.03–0.12	100	0	0
Ertapenem	0.12	0.12	0.1	0.03–0.12	100	0	0
Imipenem	0.12	0.25	0.1	0.06–0.25	100	0	0
Meropenem	0.062	0.12	0.1	0.03–0.12	100	0	0
Ceftriaxone	>32	>32	13.4	0.03–>32	10	47	43
Levofloxacin	1	4	1.1	0.25–16	86	9	5
Fusobacterium species (15)							
Doripenem	0.031	1	0.1	0.03–1	100	0	0
Ertapenem	0.031	1	0	0.03–>32	93	0	7
Imipenem	0.12	1	0.1	0.03–2	100	0	0
Meropenem	0.031	0.12	0	0.03–0.50	100	0	0
Ceftriaxone	0.5	32	0.6	0.06–>32	87	6	7
Levofloxacin	1	1	0.8	0.25–1	100	0	0
Porphyromonas species (20)							
Doripenem	0.031	0.5	0.1	0.03–4	100	0	0
Ertapenem	0.031	0.5	0.1	0.03–32	95	0	5
Imipenem	0.031	0.12	0.1	0.03–1	100	0	0
Meropenem	0.031	0.5	0.1	0.03–8	95	5	0
Ceftriaxone	0.12	1	0.2	0.03–>32	95	0	5
Levofloxacin	0.25	1	0.4	0.06–2	100	0	0
Prevotella oris/buccae (10)							
Doripenem	0.12	0.5	0.2	0.03–0.50	100	0	0
Ertapenem	0.12	0.25	0.2	0.06–0.50	100	0	0
Imipenem	0.062	0.25	0.1	0.03–0.50	100	0	0
Meropenem	0.12	0.25	0.1	0.03–0.25	100	0	0
Ceftriaxone	8	>32	3.3	0.50–>32	50	20	30
Levofloxacin	1	1	1.1	0.50–4	90	10	0
Prevotella bivia and *disiens* (15)							
Doripenem	0.12	0.5	0.1	0.03–4	100	0	0
Ertapenem	0.25	0.5	0.2	0.03–1	100	0	0
Imipenem	0.062	0.25	0.1	0.03–2	100	0	0

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TABLE 1—*Continued*

Organism (n^b) and antimicrobial agent	MIC_{50}	MIC_{90}	Geometric mean MIC	Range	%S	%I	%R
Meropenem	0.12	0.5	0.1	0.03–2	100	0	0
Ceftriaxone	2	32	1.9	0.12–>32	87	6	7
Levofloxacin	1	8	1.4	0.25–8	73	7	20
Prevotella intermedia/nigrescens (10)							
Doripenem	0.031	0.062	0	0.03–0.06	100	0	0
Ertapenem	0.031	0.062	0	0.03–0.06	100	0	0
Imipenem	0.031	0.031	0	0.03–0.03	100	0	0
Meropenem	0.062	0.062	0.1	0.03–0.06	100	0	0
Ceftriaxone	0.12	4	0.3	0.03–8	100	0	0
Levofloxacin	0.5	0.5	0.5	0.50–0.50	100	0	0
Other *Prevotella* species (22)							
Doripenem	0.062	0.12	0.1	0.03–0.25	100	0	0
Ertapenem	0.062	0.25	0.1	0.03–0.25	100	0	0
Imipenem	0.031	0.062	0	0.03–0.12	100	0	0
Meropenem	0.062	0.25	0.1	0.03–0.25	100	0	0
Ceftriaxone	0.25	16	0.4	0.06–>32	95	0	5
Levofloxacin	1	1	0.9	0.50–1	100	0	0
Sutterella wadsworthensis (12)							
Doripenem	4	8	2.3	0.06–32	73	18	9
Ertapenem	0.5	1	0.3	0.03–1	100	0	0
Imipenem	1	4	1.2	0.03–16	91	0	9
Meropenem	4	4	1.1	0.03–>32	92	0	8
Ceftriaxone	>32	>32	3.2	0.25–>32	25	0	75
Levofloxacin	0.5	2	0.7	0.25–2	100	0	0
Clostridium species (25)							
Doripenem	1	2	0.5	0.03–4	100	0	0
Ertapenem	2	8	0.8	0.03–8	84	16	0
Imipenem	2	8	0.9	0.03–8	80	20	0
Meropenem	1	2	0.5	0.03–4	100	0	0
Ceftriaxone	8	32	5.3	0.25–>32	72	24	4
Levofloxacin	4	32	4	0.25–32	28	28	44
Anaerobic gram-positive cocci (18)							
Doripenem	0.031	0.12	0	0.03–0.25	100	0	0
Ertapenem	0.031	0.25	0.1	0.03–0.25	100	0	0
Imipenem	0.062	0.062	0	0.03–0.12	100	0	0
Meropenem	0.031	0.12	0	0.03–0.12	100	0	0
Ceftriaxone	0.25	4	0.4	0.12–4	100	0	0
Levofloxacin	0.5	2	0.5	0.25–8	95	0	5
Nonsporing gram-positive rods (30)							
Doripenem	0.12	0.25	0.2	0.03–8	97	3	0
Ertapenem	0.12	0.5	0.2	0.03–16	97	0	3
Imipenem	0.12	0.25	0.1	0.03–1	100	0	0
Meropenem	0.062	0.25	0.1	0.03–8	97	3	0
Ceftriaxone	0.25	0.5	0.2	0.06–16	100	0	0
Levofloxacin	2	8	1.7	0.25–32	80	7	13
Total (364)							
Doripenem (424)	0.25	1	0.3	0.03–32	98	1	1
Ertapenem	0.25	1	0.2	0.03–>32	98	1	1
Imipenem	0.12	1	0.2	0.03–16	98	2	0
Meropenem	0.12	0.5	0.2	0.03–>32	99	1	0
Ceftriaxone	32	>32	3	0.03–>32	47	29	24
Levofloxacin	1	8	1.7	0.06–>32	69	11	20

[a] %S, %I, and %R, percentage of isolates susceptible, intermediate, or resistant, respectively; grp., group. Susceptible (intermediate) breakpoints used were as follows: for ertapenem, imipenem, and meropenem, 4 (8) μg/ml; and for ceftriaxone, 16 (32) μg/ml. Doripenem does not have a CLSI-approved breakpoint, and a tentative breakpoint of 4 (8) μg/ml was used to facilitate comparison with the other carbapenems. A tentative breakpoint of 2 (4) μg/ml was used for levofloxacin, which also does not have a CLSI-approved breakpoint.

[b] No. of isolates.

[c] No. of isolates tested with doripenem.

≤4 μg/ml, except levofloxacin. Several strains of *Prevotella* species were resistant to ≥32 μg/ml of ceftriaxone.

Results for strains of *Bilophila wadsworthia* are notoriously difficult to read in MIC tests. Others have reported higher MICs of carbapenems and other β-lactams for *B. wadsworthia* (4). We believe that this is due to the heavy haze seen on MIC plates; we have shown that the haze is composed of cell wall-deficient forms (15). We do not know whether the cell wall-active forms can persist in vivo or whether they have any clinical significance. MICs were redetermined with the use of TTC (a viable dye) (14). Using TTC endpoints, none of the *Bilophila* strains was resistant to any of the carbapenems. Doripenem and the other carbapenems had high MICs for a few of the *Sutterella* strains; results for these strains are also difficult to read and were retested in the presence of formate/fumarate (an additive that enhances growth and makes MICs easier to read), but this did not appreciably affect the MICs.

All of the carbapenems were active against *Clostridium* species other than *C. difficile.* Several strains of these species were resistant to levofloxacin. Three to five of the six strains of *C. difficile* tested required 8 μg/ml of ertapenem, imipenem, or meropenem for inhibition; all but one strain of *C. difficile* was inhibited by 2 μg/ml of doripenem (that strain was inhibited by 4 μg/ml.)

Gram-positive cocci (*Finegoldia magna* [formerly *Peptostreptococcus magnus*], *Micromonas micros* [formerly *Peptostreptococcus micros*], and *Ruminococcus* species) were all inhibited by ≤4 μg/ml of all the agents tested, except for one strain of *Ruminococcus* species, for which levofloxacin had an MIC of 8 μg/ml. All of the agents except levofloxacin were active against non-spore-forming gram-positive rods, except for one strain of *Actinomyces* sp., for which all of the carbapenem agents except imipenem had MICs of 8 to 16 μg/ml (imipenem had an MIC of 1 μg/ml for this strain).

In other studies, doripenem showed greater activity than meropenem did against gram-positive cocci and greater activity than imipenem did against gram-negative rods and was more active than both of those carbapenems against *Pseudomonas aeruginosa* (17). Doripenem was either similar to or more active than imipenem, meropenem, and biapenem against gram-positive bacteria (18) from respiratory infections and was the most potent agent against *P. aeruginosa* (17, 18). Doripenem was active against various urological (9) and gynecological (6) pathogens.

In more-recent studies presented at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, doripenem displayed potent in vitro activity against *Enterobacteriaceae* and *Acinetobacter baumanii* (except those with carbapenemases) and activities similar to those of other carbapenems to common gram-positive pathogens (Y. Ge, R. S. Blosser, J. A. Karlowsky, and D. F. Sahm, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-2008, 2003). Doripenem retains activity against many β-lactamase-producing *Enterobacteriaceae* and against fluoroquinolone and macrolide-resistant gram-positive bacteria (Y. Ge et al., 43rd ICAAC, abstr. E-2008; D. M. Livermore, S. Mushtaq, M. Warner, and Y. Ge, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-529, 2003). In another study, doripenem was active against all anaerobes tested (MIC range, 0.015 to 4 μg/ml) (R. N. Jones, H. Huyn, and D. J. Biedenbach, 43rd ICAAC, abstr. F-527, 2003). Like meropenem and unlike imipenem, the MICs of doripenem were somewhat elevated in *Pseudomonas aeruginosa* strains with enhanced efflux expression (D. M. Livermore, S. Mushtaq, M. Warner, and Y. Ge, 43rd ICAAC, abstr. F-530, 2003), indicating that doripenem is a substrate of the MexAB-OprM pump. Our data (above) also suggest that doripenem is a substrate of efflux pumps.

In summary, doripenem appears to have excellent activity against a broad range of anaerobes and better activity than the other carbapenems against *C. difficile*. The only exception was *Sutterella wadsworthensis*. Doripenem's activity is similar to those of ertapenem, meropenem, and imipenem. Further studies of the clinical usefulness of this agent for anaerobic and mixed aerobic-anaerobic infections are warranted.

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REFERENCES

1. **Bandoh, K., K. Ueno, K. Watanabe, and N. Kato.** 1993. Susceptibility patterns and resistance to imipenem in the *Bacteroides fragilis* group species in Japan: a 4-year study. Clin. Infect. Dis. **16**(Suppl. 4):s382—s386.
2. **Edwards, R., and P. N. Read.** 2000. Expression of the carbapenemase gene (*cfiA*) in *Bacteroides fragilis*. J. Antimicrob. Chemother. **46:**1009–1012.
3. **Gimeno, C., D. Navarro, F. Savall, E. Millas, M. A. Farga, J. Garau, R. Cisterna, and J. Garcia-de-Lomas.** 1996. Relationship between outer membrane protein profiles and resistance to ceftazidime, imipenem, and ciprofloxacin in *Pseudomonas aeruginosa* isolates from bacteremic patients. Eur. J. Clin. Microbiol. Infect. Dis. **15:**82–85.
4. **Goldstein, E. J., D. M. Citron, M. C. Vreni, Y. Warren, and K. L. Tyrrell.** 2000. Comparative in vitro activities of ertapenem (MK-0826) against 1,001 anaerobes isolated from human intra-abdominal infections. Antimicrob. Agents Chemother. **44:**2389–2394.
5. **Jousimies-Somer, H., P. Summanen, D. M. Citron, E. J. Baron, H. M. Wexler, and S. M. Finegold.** 2002. Wadsworth-KTL anaerobic bacteriology manual. Star Publishing Company, Belmont, Calif.
6. **Mikamo, H., K. Izumi, Y. X. Hua, Y. Hayasaki, Y. Sato, and T. Tamaya.** 2000. In vitro and in vivo antibacterial activities of a new injectable carbapenem, S-4661, against gynaecological pathogens. J. Antimicrob. Chemother. **46:**471–474.
7. **Mori, M., M. Hikida, T. Nishihara, T. Nasu, and S. Mitsuhashi.** 1996. Comparative stability of carbapenem and penem antibiotics to human recombinant dehydropeptidase-I. J. Antimicrob. Chemother. **37:**1034–1036.
8. **National Committee for Clinical Laboratory Standards.** 2003. Methods for antimicrobial susceptibility testing of anaerobic bacteria: approved standard, 6th ed. NCCLS document M11–A6. NCCLS, Wayne, Pa.
9. **Nomura, S., and A. Nagayama.** 2002. In vitro antibacterial activity of S-4661, a new parenteral carbapenem, against urological pathogens isolated from patients with complicated urinary tract infections. J. Chemother. **14:**155–160.
10. **Ochs, M. M., M. Bains, and R. E. Hancock.** 2000. Role of putative loops 2 and 3 in imipenem passage through the specific porin OprD of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **44:**1983–1985.
11. **Okamoto, K., N. Gotoh, and T. Nishino.** 2001. *Pseudomonas aeruginosa* reveals high intrinsic resistance to penem antibiotics: penem resistance mechanisms and their interplay. Antimicrob. Agents Chemother. **45:**1964–1971.
12. **Pfaller, M. A., and R. N. Jones.** 1997. A review of the in vitro activity of meropenem and comparative antimicrobial agents tested against 30,254 aerobic and anaerobic pathogens isolated world wide. Diagn. Microbiol. Infect. Dis. **28:**157–163.
13. **Podglajen, I., J. Breuil, F. Bordon, L. Gutmann, and E. Collatz.** 1992. A silent carbapenemase gene in strains of Bacteroides fragilis can be expressed after a one-step mutation. FEMS Microbiol. Lett. **70:**21–29.
14. **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.
15. **Summanen, P., H. M. Wexler, K. W. Lee, S. Becker, M. Garcia, and S. M. Finegold.** 1993. The morphological response of *Bilophila wadsworthia* to imipenem: correlation with the properties of penicillin-binding proteins. Antimicrob. Agents Chemother. **37:**2638–2644.
16. **Tanaka-Bandoh, K., N. Kato, K. Watanabe, and K. Ueno.** 1995. Antibiotic susceptibility profiles of Bacteroides fragilis and Bacteroides thetaiotaomicron in Japan from 1990 to 1992. Clin. Infect. Dis. **20**(Suppl. 2)**:**S352—S355.

17. **Tsuji, M., Y. Ishii, A. Ohno, S. Miyazaki, and K. Yamaguchi.** 1998. In vitro and in vivo antibacterial activities of S-4661, a new carbapenem. Antimicrob. Agents Chemother. **42:**94–99.
18. **Watanabe, A., H. Takahashi, T. Kikuchi, T. Kobayashi, K. Gomi, S. Fujimura, Y. Tokue, and T. Nukiwa.** 2000. Comparative in vitro activity of S-4661, a new parenteral carbapenem, and other antimicrobial agents against respiratory pathogens. Chemotherapy **46:**184–187.
19. **Wexler, H. M., E. Molitoris, F. Jashnian, and S. M. Finegold.** 1991. Comparison of spiral gradient with conventional agar dilution for susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. **35:**1196–1202.
20. **Wexler, H. M., E. Molitoris, P. R. Murray, J. Washington, R. J. Zabransky, P. H. Edelstein, and S. M. Finegold.** 1996. Comparison of spiral gradient endpoint and agar dilution methods for susceptibility testing of anaerobic bacteria: a multilaboratory collaborative evaluation. J. Clin. Microbiol. **34:** 170–174.