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Antimicrobial Susceptibility Patterns for Recent Clinical Isolates of Anaerobic Bacteria in South Korea[▽]

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We determined the antimicrobial susceptibilities of 255 clinical isolates of anaerobic bacteria collected in 2007 and 2008 at a tertiary-care hospital in South Korea. Piperacillin-tazobactam, cefoxitin, imipenem, and meropenem were highly active β-lactam agents against most of the isolates tested. The rates of resistance of *Bacteroides fragilis* group organisms and anaerobic Gram-positive cocci to moxifloxacin were 11 to 18% and 0 to 27%, respectively.

Anaerobic bacterial resistance trends may vary greatly, depending on regions or institutions (1). The Clinical and Laboratory Standards Institute (CLSI) does not recommend routine susceptibility testing of all clinical isolates of anaerobic bacteria, except for the management of patients with serious infections (4). A recent survey indicated that only a few laboratories in the United States performed antimicrobial susceptibility testing of anaerobic bacteria due to the complex techniques and predictable susceptibilities involved (5). However, regional susceptibility patterns are pivotal in the empirical treatment of infected patients because these patterns are related to clinical outcomes (13). Therefore, periodic monitoring of the regional or institutional resistance trends of clinically important anaerobe isolates is recommended (4). Our investigation of resistance trends of *Bacteroides fragilis* group organisms from South Korea has been taking place since 1989 (9, 15). However, few studies have focused on the susceptibilities of other anaerobes. Therefore, the aim of this study was to determine the recent antimicrobial resistance patterns of frequently isolated anaerobes at a tertiary-care hospital in South Korea.

Anaerobes were isolated from blood, normally sterile body fluid, and abscess specimens, but *Clostridium difficile* was isolated from stool specimens of suspected *C. difficile*-associated disease patients at Severance Hospital in 2007 and 2008. The isolates were identified by either conventional methods (19) or the ATB 32A system (bioMérieux, Marcy l'Etoile, France). A total of 255 nonduplicated isolates were used in this study, including 63 of *B. fragilis*, 57 of other *B. fragilis* group species, 28 of *Prevotella* spp., 9 of other Gram-negative bacilli, 15 of *Anaerococcus prevotii*, 15 of *Peptoniphilus asaccharolyticus*, 15 of *Finegoldia magna*, 13 of *Peptostreptococcus* spp., 15 of *C. perfringens*, 12 of *C. difficile*, and 13 of other Gram-positive bacilli.

Antimicrobial susceptibility testing was performed using the CLSI agar dilution method (4). The medium used was *Brucella* agar (Becton Dickinson, Cockeysville, MD) supplemented with 5 μg hemin and 1 μg vitamin K_1 per ml and 5% laked sheep blood. The antimicrobial powders used were piperacillin and tazobactam (Yuhan, Seoul, South Korea), cefoxitin (Merck Sharp & Dohme, West Point, PA), cefotetan (Daiichi Pharmaceutical, Tokyo, Japan), clindamycin (Korea Upjohn, Seoul, South Korea), imipenem, metronidazole (Choong Wae, Seoul, South Korea), chloramphenicol (Chong Kun Dang, Seoul, South Korea), meropenem (Sumitomo, Tokyo, Japan), moxifloxacin (Bayer Korea, Seoul, South Korea), and vancomycin (Eli Lilly & Co., Indianapolis, IN). For the combination of piperacillin and tazobactam, a constant tazobactam concentration of 4 μg/ml was added.

An inoculum of 10^5 CFU was applied with a Steers replicator (Craft Machine Inc., Woodline, PA), and the plates were incubated in an anaerobic chamber (Forma Scientific, Marietta, OH) for 48 h at 37°C. The MIC of each antimicrobial agent was defined as the concentration at which there was a marked reduction in growth, such as from confluent colonies to a haze, <10 tiny colonies, or 1 to 3 normal-sized colonies. *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were used as controls.

β-Lactamase production by anaerobic Gram-negative bacilli, with the exception of *B. fragilis* group organisms, was determined by applying test organisms to the Cefinase disks and recording the results after 30 min (Becton Dickinson, Cockeysville, MD).

Table 1 shows the MICs of antimicrobial agents and the resistance rates of the anaerobes tested. Among the 255 isolates, *B. fragilis* group organisms were the most prevalent (47%). These organisms are more virulent and more resistant to antimicrobial agents than most other anaerobes (3). In this study, piperacillin-tazobactam, cefoxitin, imipenem, and meropenem were highly active against *B. fragilis* group organisms, with resistance rates of less than 7%. The rates of resistance to imipenem and piperacillin-tazobactam were 4% and 7%, respectively, for other *B. fragilis* group organisms. However, much higher resistance rates were observed for piperacillin (27

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TABLE 1. Antimicrobial activities against 255 anaerobic bacteria isolated in 2007 to 2008

Organism (no. of isolates) and antimicrobial agent	Breakpoint (μg/ml)[a]			MIC (μg/ml)			Susceptibility (%)[a]		
	S	I	R	Range	50%	90%	S	I	R
Bacteroides fragilis (63)									
Piperacillin	≤32	64	≥128	4–>256	8	>256	67	6	27
Piperacillin-tazobactam	≤32	64	≥128	0.25–128	1	4	97	2	2
Cefoxitin	≤16	32	≥64	8–128	16	32	79	16	5
Cefotetan	≤16	32	≥64	4–>128	8	64	71	14	14
Imipenem	≤4	8	≥16	0.06–32	0.125	1	98	0	2
Meropenem	≤4	8	≥16	0.06–128	0.125	4	92	5	3
Clindamycin	≤2	4	≥8	≤0.06–>128	0.5	>128	67	0	33
Moxifloxacin	≤2	4	≥8	0.25–>128	0.5	8	84	5	11
Chloramphenicol	≤8	16	≥32	2–16	4	4	98	2	0
Metronidazole	≤8	16	≥32	0.5–8	2	2	100	0	0
B. fragilis group, other species (57)[b]									
Piperacillin	≤32	64	≥128	8–>256	128	>256	42	7	51
Piperacillin-tazobactam	≤32	64	≥128	1–>128	8	64	89	4	7
Cefoxitin	≤16	32	≥64	4–>128	32	32	25	68	7
Cefotetan	≤16	32	≥64	4–>128	>128	>128	89	5	86
Imipenem	≤4	8	≥16	0.13–32	0.5	4	95	2	4
Meropenem	≤4	8	≥16	0.13–8	0.25	2	98	2	0
Clindamycin	≤2	4	≥8	0.06–>128	>128	>128	16	16	68
Moxifloxacin	≤2	4	≥8	0.13–>128	2	16	72	10	18
Chloramphenicol	≤8	16	≥32	4–16	4	8	98	2	0
Metronidazole	≤8	16	≥32	0.5–4	2	2	100	0	0
Prevotella intermedia (10)									
Piperacillin	≤32	64	≥128	2–16	8	16	100	0	0
Piperacillin-tazobactam	≤32	64	≥128	≤0.03	≤0.03	≤0.03	100	0	0
Cefoxitin	≤16	32	≥64	0.5–4	2	4	100	0	0
Cefotetan	≤16	32	≥64	0.13–16	2	16	100	0	0
Imipenem	≤4	8	≥16	0.02–0.06	0.03	0.06	100	0	0
Meropenem	≤4	8	≥16	0.03–0.06	0.06	0.06	100	0	0
Clindamycin	≤2	4	≥8	≤0.06–2	≤0.06	≤0.06	100	0	0
Moxifloxacin	≤2	4	≥8	0.5	0.5	0.5	100	0	0
Chloramphenicol	≤8	16	≥32	0.5–1	0.5	1	100	0	0
Metronidazole	≤8	16	≥32	0.5–2	0.5	2	100	0	0
Prevotella spp. (18)[c]									
Piperacillin	≤32	64	≥128	0.5–256	16	128	78	11	11
Piperacillin-tazobactam	≤32	64	≥128	≤0.03–16	≤0.03	4	100	0	0
Cefoxitin	≤16	32	≥64	0.5–32	1	32	89	11	0
Cefotetan	≤16	32	≥64	0.5–64	4	64	72	11	17
Imipenem	≤4	8	≥16	0.03–1	0.06	0.5	100	0	0
Meropenem	≤4	8	≥16	0.03–1	0.125	0.5	100	0	0
Clindamycin	≤2	4	≥8	≤0.06–128	≤0.06	128	50	0	50
Moxifloxacin	≤2	4	≥8	0.5–16	2	8	56	33	11
Chloramphenicol	≤8	16	≥32	0.5–8	4	8	100	0	0
Metronidazole	≤8	16	≥32	0.5–8	4	8	100	0	0
Other Gram-negative bacilli (9)[d]									
Piperacillin	≤32	64	≥128	0.06–32	NA[g]	NA	NA	NA	NA
Piperacillin-tazobactam	≤32	64	≥128	≤0.03–4	NA	NA	NA	NA	NA
Cefoxitin	≤16	32	≥64	≤0.06–8	NA	NA	NA	NA	NA
Cefotetan	≤16	32	≥64	≤0.06–8	NA	NA	NA	NA	NA
Imipenem	≤4	8	≥16	0.02–4	NA	NA	NA	NA	NA
Meropenem	≤4	8	≥16	≤0.008–4	NA	NA	NA	NA	NA
Clindamycin	≤2	4	≥8	≤0.06–128	NA	NA	NA	NA	NA
Moxifloxacin	≤2	4	≥8	0.25–128	NA	NA	NA	NA	NA
Chloramphenicol	≤8	16	≥32	0.13–4	NA	NA	NA	NA	NA
Metronidazole	≤8	16	≥32	0.13–1	NA	NA	NA	NA	NA
Peptostreptococcus spp. (13)[e]									
Piperacillin	≤32	64	≥128	0.06–16	0.25	16	100	0	0
Piperacillin-tazobactam	≤32	64	≥128	≤0.03–16	0.25	16	100	0	0
Cefoxitin	≤16	32	≥64	0.25–16	1	16	100	0	0
Cefotetan	≤16	32	≥64	≤0.06–128	4	64	62	8	31

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TABLE 1—*Continued*

Organism (no. of isolates) and antimicrobial agent	Breakpoint (μg/ml)[a]			MIC (μg/ml)			Susceptibility (%)[a]		
	S	I	R	Range	50%	90%	S	I	R
Imipenem	≤4	8	≥16	≤0.008–4	0.125	2	100	0	0
Meropenem	≤4	8	≥16	0.01–4	0.25	4	100	0	0
Clindamycin	≤2	4	≥8	≤0.06–128	0.125	64	77	0	23
Moxifloxacin	≤2	4	≥8	≤0.06–8	0.125	0.25	92	0	8
Chloramphenicol	≤8	16	≥32	1–2	2	2	100	0	0
Metronidazole	≤8	16	≥32	0.25–1	0.5	1	100	0	0
Anaerococcus prevotii (15)									
Piperacillin	≤32	64	≥128	≤0.06–0.5	0.125	0.25	100	0	0
Piperacillin-tazobactam	≤32	64	≥128	≤0.03–1	0.125	0.125	100	0	0
Cefoxitin	≤16	32	≥64	≤0.06–4	0.5	1	100	0	0
Cefotetan	≤16	32	≥64	≤0.06–4	1	2	100	0	0
Imipenem	≤4	8	≥16	≤0.008–0.25	0.06	0.25	100	0	0
Meropenem	≤4	8	≥16	≤0.008–0.25	0.06	0.125	100	0	0
Clindamycin	≤2	4	≥8	≤0.06–128	2	128	60	0	40
Moxifloxacin	≤2	4	≥8	≤0.06–8	0.25	8	87	0	13
Chloramphenicol	≤8	16	≥32	1–16	4	8	93	7	0
Metronidazole	≤8	16	≥32	0.25–1	1	1	100	0	0
Peptoniphilus asaccharolyticus (15)									
Piperacillin	≤32	64	≥128	≤0.06–0.25	≤0.06	≤0.06	100	0	0
Piperacillin-tazobactam	≤32	64	≥128	≤0.03–0.25	≤0.03	0.06	100	0	0
Cefoxitin	≤16	32	≥64	≤0.06–1	≤0.06	0.5	100	0	0
Cefotetan	≤16	32	≥64	0.13–2	0.25	1	100	0	0
Imipenem	≤4	8	≥16	≤0.008–0.13	≤0.008	0.03	100	0	0
Meropenem	≤4	8	≥16	≤0.008–0.06	≤0.008	0.03	100	0	0
Clindamycin	≤2	4	≥8	≤0.06–32	0.125	32	67	0	33
Moxifloxacin	≤2	4	≥8	0.13–2	0.25	2	100	0	0
Chloramphenicol	≤8	16	≥32	1–4	2	4	100	0	0
Metronidazole	≤8	16	≥32	0.5–2	1	1	100	0	0
Finegoldia magna (15)									
Piperacillin	≤32	64	≥128	≤0.06–0.25	≤0.06	0.125	100	0	0
Piperacillin-tazobactam	≤32	64	≥128	≤0.03–0.25	0.06	0.125	100	0	0
Cefoxitin	≤16	32	≥64	≤0.06–1	0.5	1	100	0	0
Cefotetan	≤16	32	≥64	0.12–4	1	2	100	0	0
Imipenem	≤4	8	≥16	≤0.008–0.13	0.06	0.125	100	0	0
Meropenem	≤4	8	≥16	0.03–0.13	0.06	0.125	100	0	0
Clindamycin	≤2	4	≥8	≤0.06–128	0.25	64	73	13	13
Moxifloxacin	≤2	4	≥8	0.13–32	0.5	8	60	13	27
Chloramphenicol	≤8	16	≥32	2–4	4	4	100	0	0
Metronidazole	≤8	16	≥32	0.5–1	0.5	1	100	0	0
Clostridium perfringens (15)									
Piperacillin	≤32	64	≥128	≤0.06–0.5	0.25	0.5	100	0	0
Piperacillin-tazobactam	≤32	64	≥128	≤0.03–1	0.25	0.5	100	0	0
Cefoxitin	≤16	32	≥64	0.5–2	1	2	100	0	0
Cefotetan	≤16	32	≥64	≤0.06–1	0.25	1	100	0	0
Imipenem	≤4	8	≥16	0.03–0.25	0.125	0.125	100	0	0
Meropenem	≤4	8	≥16	≤0.008–0.03	0.015	0.015	100	0	0
Clindamycin	≤2	4	≥8	≤0.06–128	2	4	80	13	7
Moxifloxacin	≤2	4	≥8	0.25–16	0.5	0.5	93	0	7
Chloramphenicol	≤8	16	≥32	2–8	4	4	100	0	0
Metronidazole	≤8	16	≥32	0.02–0.06	0.03	0.06	100	0	0
Vancomycin	NA	NA	NA	0.25–1	0.5	0.5	NA	NA	NA
Clostridium difficile (12)									
Piperacillin	≤32	64	≥128	2–8	4	8	100	0	0
Piperacillin-tazobactam	≤32	64	≥128	1–16	4	8	100	0	0
Cefoxitin	≤16	32	≥64	64–>128	64	>128	0	0	100
Cefotetan	≤16	32	≥64	8–128	8	128	83	0	17
Imipenem	≤4	8	≥16	0.25–16	4	8	58	33	8
Meropenem	≤4	8	≥16	0.25–2	2	2	100	0	0
Clindamycin	≤2	4	≥8	2–128	64	128	8	8	83
Moxifloxacin	≤2	4	≥8	1–>128	16	32	25	0	75
Chloramphenicol	≤8	16	≥32	1–16	4	16	83	17	0

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TABLE 1—*Continued*

Organism (no. of isolates) and antimicrobial agent	Breakpoint (μg/ml)[a]			MIC (μg/ml)			Susceptibility (%)[a]		
	S	I	R	Range	50%	90%	S	I	R
Metronidazole	≤8	16	≥32	0.5–2	1	1	100	0	0
Vancomycin	NA	NA	NA	0.25–2	0.5	2	NA	NA	NA
Other Gram-positive bacilli (13)[f]									
Piperacillin	≤32	64	≥128	≤0.06–64	1	8	92	8	0
Piperacillin-tazobactam	≤32	64	≥128	≤0.03–64	0.5	8	92	8	0
Cefoxitin	≤16	32	≥64	0.13–32	2	16	92	8	0
Cefotetan	≤16	32	≥64	0.13–>128	4	64	85	0	15
Imipenem	≤4	8	≥16	≤0.008–2	0.06	0.5	100	0	0
Meropenem	≤4	8	≥16	≤0.008–16	0.125	2	92	0	8
Clindamycin	≤2	4	≥8	≤0.06–>128	0.06	>128	85	0	15
Moxifloxacin	≤2	4	≥8	≤0.06–4	1	2	92	8	0
Chloramphenicol	≤8	16	≥32	1–16	1	2	92	8	0
Metronidazole	≤8	16	≥32	0.5–>128	16	>128	46	8	46

[a] S, susceptible; I, intermediate; R, resistant.
[b] *Bacteroides thetaiotaomicron* ($n = 25$), *B. caccae* ($n = 3$), *B. distasonis* ($n = 9$), *B. ovatus* ($n = 8$), and *B. vulgatus* ($n = 12$).
[c] *Prevotella bivia* ($n = 10$), *P. buccae* ($n = 5$), and *P. oralis* ($n = 3$).
[d] *Porphyromonas asaccharolytica* ($n = 2$), *Fusobacterium varium* ($n = 3$), *F. necrogenes* ($n = 2$), *F. nucleatum* ($n = 1$), and *F. mortiferum* ($n = 1$).
[e] *Peptostreptococcus anaerobius* ($n = 9$) and *P. micros* ($n = 4$).
[f] *Acitnomyces odontolyticus* ($n = 3$), *A. israelii* ($n = 2$), *A. meyeri* ($n = 1$), *A. naeslundii* ($n = 1$), *Bifidobacterium adolescentis* ($n = 3$), *Bifidobacterium* sp. ($n = 1$), *Eubacterium lentum* ($n = 1$), and *Eubacterium* sp. ($n = 1$).
[g] NA, not available/not applicable.

to 51%), cefotetan (14 to 68%), and clindamycin (33 to 86%). These values were similar to those observed in 1997 to 2004 in the same hospital: piperacillin, 33 to 49%; cefotetan, 14 to 60%; clindamycin, 51 to 76% (15). A higher prevalence of resistance, in particular to clindamycin, was observed than in the United States, i.e.,19 to 35% (17). CLSI added a recommendation to test susceptibility to moxifloxacin in 2004 and 2007. In this study, the moxifloxacin resistance rates were 11% for *B. fragilis* and 18% for other *B. fragilis* group organisms. These rates were slightly higher than the 7 to 9% reported in Taiwan (11) but lower than those in Greece (14) and the United States (16 to 75% and 26 to 55%, respectively) (17).

Overall, *Prevotella*, *Porphyromonas*, and *Fusobacterium* isolates are more susceptible than *B. fragilis* group organisms (7). Among these organisms, β-lactamase producers were resistant to penicillin and ampicillin (3, 7). A recent study showed that 94% of the *Prevotella* isolates tested were β-lactamase producers, which correlated well with susceptibility to penicillin (11). In the present study, β-lactamase production was detected in 26 *Prevotella* isolates (94%) and 1 *Fusobacterium* isolate (14%). While 50% of the non-*P. intermedia Prevotella* isolates were resistant to clindamycin, all of the *P. intermedia* isolates were susceptible to clindamycin. Other studies indicated that 17% and 36% of the *P. intermedia* isolates were resistant to clindamycin (8, 16).

Anaerobic Gram-positive cocci account for approximately one-quarter of all isolates from anaerobic infections. They may cause various infections, including skin infections, necrotizing pneumonia, and bacteremia (18). Several species previously placed in the genus *Peptostreptococcus* have been reclassified into new genera, including *Anaerococcus*, *Finegoldia*, *Micrococcus*, and *Peptoniphilus* (7). These organisms exhibited various rates of resistance to penicillin, clindamycin, and metronidazole (7). A European surveillance study showed that the majority of the isolates found to be resistant to clindamycin and penicillin were identified as *F. magna* (2). In our study, the rates of resistance of Gram-positive cocci to clindamycin and moxifloxacin varied according to species. The highest clindamycin resistance observed was 40% of *A. prevotii* isolates, followed by 33% of *P. asaccharolyticus* isolates. These rates were much higher than those reported in Europe (4%) and the United States (8%) (1, 2) but similar to the 25.9% observed in 1994 in South Korea (10). The rates of resistance to moxifloxacin varied from 27% among *F. magna* isolates to 0% among *P. asaccharolyticus* isolates. The difference in resistance rates among anaerobic Gram-positive cocci may be of importance. The resistance patterns of these organisms could help in the selection of appropriate antimicrobial treatment options, although susceptibility testing of individual patient isolates is not performed.

C. perfringens is generally very susceptible to most antibiotics (7). The present study showed that all of the antimicrobial agents tested had high activity against this organism. *C. difficile* has highly variable resistance to β-lactams, including penicillin, cephalosporins, imipenem, clindamycin, and moxifloxacin (6, 7). In our study, the rates of resistance to cefoxitin, clindamycin, and moxifloxacin were 100%, 85%, and 77%, respectively. The *C. difficile* NAP1/027 epidemic isolates were known to be resistant to moxifloxacin (12). A high rate of resistance to moxifloxacin was observed in this study, although none of the isolates were NAP1/027 strains. Other Gram-positive bacilli, such as *Actinomyces*, *Bifidobacterium*, and *Eubacterium* species, are generally susceptible to β-lactams, including penicillin. Metronidazole-resistant isolates were common among these organisms (3). In our study, 46% of these organisms were resistant to metronidazole.

In conclusion, piperacillin-tazobactam, cefoxitin, imipenem, meropenem, metronidazole, and chloramphenicol remain active against most anaerobic isolates. The rates of resistance of Gram-positive cocci to clindamycin and moxifloxacin are variable according to species. The rates of resistance to moxifloxacin are as follows: *C. difficile*, 75%; anaerobic Gram-positive

cocci, 0 to 27%; *B. fragilis* group organisms, 11 to 18%. Continuous monitoring is necessary to detect pattern changes at regional centers.

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