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High Incidence of Cefoxitin and Clindamycin Resistance among Anaerobes in Taiwan

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Susceptibilities to 16 antimicrobial agents were determined by measurement of MICs for 344 isolates of anaerobic bacteria recovered from patients with significant infections. Resistance rates varied among antimicrobial agents and the species tested. The β-lactams were more active in gram-positive than in gram-negative anaerobes. Resistance to meropenem was low (<1%). For β-lactam–β-lactamase inhibitors, piperacillin-tazobactam was most active for all species (resistance, <6%). The rates of resistance to cefoxitin (31 to 65%) and clindamycin (50 to 70%) for non-*Bacteroides fragilis* species of the *B. fragilis* group were higher than those for *B. fragilis* (4% resistant to cefoxitin and 33% resistant to clindamycin). Among members of *B. fragilis* group, *Bacteroides thetaiotaomicron* was the most resistant to clindamycin (70%) and cefoxitin (65%). Rates of susceptibility to imipenem and metronidazole for *B. fragilis* continue to be high compared to those from a previous study 10 years ago. However, resistance to metronidazole was found recently in five strains of *B. fragilis*. We analyzed the genetic relationships among the metronidazole-resistant *B. fragilis* strains by pulsed-field gel electrophoresis. The metronidazole-resistant *B. fragilis* strains showed genotypic heterogeneity, excluding the dissemination of a single clone.

Over the past 10 years, there has been a significant problem with increasing resistance to antimicrobial agents among anaerobic bacteria (1–3, 7–9). Antimicrobial resistance is becoming less predictable and may fluctuate from one medical center to another, as well as from one geographic region to another (1–4, 7–9). The increasing resistance among several species emphasizes the need to survey the susceptibility patterns of these organisms. Data on susceptibilities of anaerobes are very limited in Taiwan except those from a previous report of 10 years ago (16). The objective of this study was to determine the susceptibility profiles of clinical isolates of anaerobes in Taiwan and to monitor susceptibility changes over time.

Organisms tested included gram-positive and gram-negative anaerobes which were clinically commonly encountered. The test antimicrobial agents included old and new agents. Among the older agents, clindamycin, cefoxitin, and piperacillin are commonly used as the initial empirical treatment for *B. fragilis* group infections. However, resistance to these agents has been shown to increase in North America, Europe, and other countries during the past decades (1–4, 6–9, 14). The 5-nitroimidazole molecules are very potent anaerobicidal agents commonly used to treat or prevent *Bacteroides* infections. Although resistance to metronidazole in *Bacteroides fragilis* strains has been reported in several countries (12), resistance to metronidazole in *B. fragilis* strains has not been reported in Taiwan before. The emergence of metronidazole-resistant *B. fragilis* strains (MIC, >32 μg/ml) in Taiwan is reported in this study.

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MATERIALS AND METHODS

Bacterial strains. A total of 344 clinical isolates of anaerobes were collected between 1998 and 2000 from the Bacteriology Laboratory, National Taiwan University Hospital, a 2,000-bed teaching hospital in northern Taiwan. These isolates were recovered from blood, pus from the intra-abdominal cavity, abscesses, soft tissue, head or neck wounds, and others. Only one isolate per patient was included.

Antimicrobial susceptibility testing. The antimicrobial agents used for susceptibility testing were as follows: penicillin, ampicillin, piperacillin, sulbactam, tazobactam, cefoperazone, clindamycin, chloramphenicol, and metronidazole (Sigma Chemical Co., St. Louis, Mo.); ticarcillin and clavulanic acid (SmithKline Beecham, Philadelphia, Pa.); cefoxitin and imipenem (Merck Sharp & Dohme, West Point, Pa.); meropenem (Sumitomo Pharmaceuticals, Osaka, Japan); cefmetazole (Sankyo, Tokyo, Japan); and moxifloxacin (Bayer Corporation, West Haven, Conn.).

Antimicrobial susceptibility was tested by an agar dilution method in accordance with guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (10). An inoculum of 10^5 CFU per well was applied with a Steers replicator onto brucella agar supplemented with vitamin K_1 and 5% pooled sheep blood. Plates were incubated in an anaerobic chamber for 48 h at 35°C. The MIC was defined as the concentration at which there was a marked change in the appearance of growth, compared with that in the control plate. Reference strains of *B. fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were used for quality control of the susceptibility tests.

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed to genotype five metronidazole-resistant *B. fragilis* strains. Each strain was grown overnight at 37°C in 10 ml of brain heart infusion broth in an anaerobic chamber. The preparation of DNA was performed as described previously (5). After appropriate preparation, the DNAs in each plug were digested with 20 U of *Xba*I (New England Biolabs, Hitchin, United Kingdom) at 37°C for 4 h. The plugs were applied to a 1% agarose gel. Electrophoresis was performed in 0.5× Tris-borate-EDTA buffer at 14°C by using a CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, Calif.), with pulse times ranging from an initial value of 4 s to a final value of 30 s, for 16 h at 200 V.

RESULTS

Antimicrobial susceptibilities. The MIC ranges, MICs at which 50% of the isolates were inhibited (MIC_{50}s), MICs at which 90% of the isolates were inhibited (MIC_{90}s), and the percentages of 344 clinical isolates of anaerobes that were susceptible, intermediate, or resistant to various antimicrobial agents are summarized in Table 1. The rates of susceptible, intermediate, and resistant isolates were determined by using the NCCLS breakpoints (10).

B. fragilis group isolates were the most encountered clinically significant isolates among the gram-negative anaerobes. The *B. fragilis* group isolates were uniformly resistant to penicillin and ampicillin. Most isolates were susceptible to imipenem or meropenem. Only one isolate of *B. fragilis* was found to have intermediate resistance to imipenem. Comparison of the susceptibilities of the individual species of the *B. fragilis* group showed that 40% of *B. fragilis* isolates were resistant to piperacillin, 44% were resistant to ticarcillin, and 17% were resistant to ampicillin-sulbactam. Four percent of *B. fragilis* isolates were resistant to cefoxitin; however, 22% had intermediate susceptibility. Other members of the *B. fragilis* group were more resistant to cefoxitin, with resistance rates between 31 and 65%. *B. thetaiotaomicron* was the species with the greatest resistance to cefoxitin (65%) and cefmetazole (80%) among the *B. fragilis* group. Resistance to clindamycin varied among the species from 33 to 70%, with the highest resistance rate occurring in *B. thetaiotaomicron* (70%), followed by *Bacteroides caccae* (67%). Chloramphenicol and metronidazole were active against >90% of isolates of the *B. fragilis* group. Only 3% of *B. fragilis* isolates were resistant to metronidazole and 2% were intermediately resistant to metronidazole.

All *Fusobacterium* isolates were susceptible to ampicillin-sulbactam, ticarcillin-clavulanic acid, cefoxitin, and imipenem. The majority of *Fusobacterium* isolates (95%) were susceptible to cefmetazole. Thirty-five percent of *Fusobacterium* isolates were resistant to clindamycin.

More than half of *Prevotella* species isolates (62%) were resistant to penicillin and ampicillin. Rates of resistance to cefoxitin, cefmetazole, and clindamycin were 6, 12, and 31%, respectively. All of the *Prevotella* isolates tested were susceptible to imipenem, meropenem, and chloramphenicol.

Among *Veillonella* isolates, 70% were resistant to penicillin and ampicillin. Five percent of *Veillonella* isolates were resistant to piperacillin, ticarcillin, piperacillin-tazobactam, and ticarcillin-clavulanic acid. Ten percent were resistant to cefoxitin, 55% were resistant to clindamycin, and 20% were resistant to metronidazole. All isolates were susceptible to ampicillin-sulbactam, imipenem, meropenem, and chloramphenicol.

Of the gram-positive isolates, the 20 *Clostridium perfringens* isolates were susceptible to all of the agents tested. Other gram-positive anaerobes showed various degrees of resistance to penicillin (12 to 16%) and ampicillin (13 to 25%). Among *Peptostreptococcus* species isolates, 16% were resistant to penicillin. All isolates were susceptible to piperacillin-tazobactam and meropenem. Of *Peptostreptococcus* species isolates, 3% were resistant to cefoxitin and imipenem but 55% were resistant to clindamycin. *Clostridium* species other than *C. perfringens* were more resistant than *C. perfringens*, with 12% of the isolates resistant to penicillin and ticarcillin, 25% resistant to ampicillin, and 31% resistant to metronidazole.

The MIC_{50}s and MIC_{90}s of moxifloxacin for all species ranged from 0.12 to 2 μg/ml (MIC_{50}s) and from 0.25 to 8 μg/ml (MIC_{90}s).

Trend of cefoxitin and clindamycin resistance in *B. thetaiotaomicron*. Figure 1 shows the annual rates of susceptibility to six routinely tested agents for all of the *Bacteroides* species isolates at National Taiwan University Hospital from 1977 to 2000. The rates of susceptibility to cefmetazole and clindamycin decreased. Susceptibility testing was performed by the disk diffusion method before 1990 and by the breakpoint agar dilution method after 1991. A stepwise increase in the rates of resistance to cefoxitin and clindamycin resistance was noted.

PFGE of metronidazole-resistant *B. fragilis* isolates. Five metronidazole-resistant strains of *B. fragilis* were found in this study. Since no metronidazole-resistant strains of *B. fragilis* were reported before in Taiwan. We analyzed the genetic relationships among these strains by PFGE. The results are shown in Fig. 2. Lanes 1 to 6 show metronidazole-nonsusceptible *B. fragilis* isolates; lanes 2 and 3 show isolates from the same individual. Lanes 7 to 9 show metronidazole-susceptible *B. fragilis* isolates. Two strains (lanes 2 or 3 and 4) were similar. Other strains have distinct patterns. These isolates showed genotypic heterogeneity, suggesting that they correspond to a highly heterogeneous population rather than to the dissemination of a single clone.

DISCUSSION

In this study the antimicrobial susceptibilities of 344 isolates of anaerobes to various agents were determined. In agreement with other reports, the susceptibility results varied among genera and species. Compared to a previous study by our group of 100 *B. fragilis* isolates done 10 years ago (16), the increased resistance of the *B. fragilis* group to cefoxitin and clindamycin is noted. Rates of resistance to cefoxitin for *B. fragilis* increased from 1 to 4% and rates of resistance to clindamycin increased from 29 to 33% over the period from 1991 to 2000. The level of chloramphenicol susceptibility remained unchanged.

As expected, the β-lactams were more active in gram-positive than in gram-negative anaerobes. According to NCCLS guidelines, members of the *B. fragilis* group are presumed to be resistant to ampicillin. *Peptostreptococcus* species have been considered fully susceptible to several β-lactam drugs, including penicillin G. In the present study, isolates resistant to penicillin within *Peptostreptococcus* species were mostly *Peptostreptococcus anaerobius*. In Korea, the rate of resistance to penicillin of *P. anaerobius* was also high, while those of other species were lower (7). Addition of a β-lactamase inhibitor generally reversed the resistance. However, *Veillonella* displayed similar susceptibilities to piperacillin and piperacillin-tazobactam and ticarcillin and ticarcillin-clavulanic acid. The rate of resistance to ampicillin-sulbactam in *B. fragilis* isolates increased from 8 (1991) to 17% (this study). Resistance to piperacillin-tazobactam was low (<6%).

Data collected from our hospital's clinical microbiology laboratory reveal the decrease in susceptibility to cefmetazole in *Bacteroides* species from 1987 to 2000. Cefoxitin was more active than cefmetazole against most species. Rates of resis-

TABLE 1. In vitro susceptibilities of clinical isolates of anaerobes

Bacterium (no. of isolates tested) and antimicrobial agent	MIC (μg/ml)			%[a]		
	Range	50%	90%	S	I	R
B. fragilis (100)						
Penicillin	2–>128	64	>128	0	0	100
Ampicillin	32–>128	>128	>128	0	0	100
Piperacillin	0.5–>128	32	>128	55	5	40
Ticarcillin	1–>128	64	>128	46	10	44
AMP-sulbactam[b]	1–64	16	32	50	33	17
Piperacillin-TAZ[c]	<0.03–16	0.5	8	100	0	0
Ticarcillin-CVA[d]	0.12–4	0.5	2	100	0	0
Cefoxitin	4–64	8	32	74	22	4
Cefmetazole	4–32	8	16	93	7	0
Cefoperazone	4–>128	128	>128	16	9	75
Imipenem	0.25–8	0.5	4	99	1	0
Meropenem	<0.03–16	0.25	8	94	5	1
Moxifloxacin	0.06–4	0.25	2			
Clindamycin	≤0.03–>128	0.25	>128	67	0	33
Chloramphenicol	1–2	2	2	100	0	0
Metronidazole	1–64	1	2	95	2	3
B. thetaiotaomicron (40)						
Penicillin	16–>128	32	>128	0	0	100
Ampicillin	32–>128	64	>128	0	0	100
Piperacillin	8–>128	128	>128	28	18	55
Ticarcillin	32–>128	8	16	5	35	60
AMP-sulbactam	2–64	16	32	53	13	35
Piperacillin-TAZ	1–64	16	32	90	10	0
Ticarcillin-CVA	0.06–64	8	16	95	5	0
Cefoxitin	16–>128	64	>128	10	25	65
Cefmetazole	8–>128	64	>128	10	10	80
Cefoperazone	64–>128	64	>128	0	0	100
Imipenem	0.12–16	0.25	4	93	0	8
Meropenem	0.12–2	0.25	2	100	0	0
Moxifloxacin	0.06–4	2	4			
Clindamycin	0.5–>128	32	>128	25	5	70
Chloramphenicol	4–8	4	4	100	0	0
Metronidazole	0.5–2	1	2	100	0	0
B. caccae (18)						
Penicillin	16–>128	128	>128	0	0	100
Ampicillin	8–>128	128	>128	0	0	100
Piperacillin	32–>128	128	>128	28	17	55
Ticarcillin	8–>128	>128	>128	11	0	89
AMP-sulbactam	8–32	16	32	11	39	50
Piperacillin-TAZ	≤0.03–>32	8	32	100	0	0
Ticarcillin-CVA	0.12–32	2	16	100	0	0
Cefoxitin	8–>128	32	>128	28	22	50
Cefmetazole	32–>128	64	>128	0	17	83
Cefoperazone	64–>128	128	>128	0	0	100
Imipenem	0.12–4	0.5	2	100	0	0
Meropenem	0.25–8	0.5	2	89	11	0
Moxifloxacin	0.25–32	1	4			
Clindamycin	0.25–>128	8	>128	33	0	67
Chloramphenicol	1–4	4	4	100	0	0
Metronidazole	0.5–16	1	2	89	11	0
B. uniformis (32)						
Penicillin	8–>128	128	>128	0	0	100
Ampicillin	32–>128	128	>128	0	0	100
Piperacillin	64–>128	128	>128	25	13	63
Ticarcillin	64–>128	>128	>128	6	0	94
AMP-sulbactam	2–32	16	32	25	12	62
Piperacillin-TAZ	0.5–>128	8	32	94	0	6
Ticarcillin-CVA	0.5–>128	2	16	88	6	6
Cefoxitin	2–>128	32	>128	38	31	31
Cefmetazole	32–>128	64	>128	0	13	88
Cefoperazone	32–>128	128	>128	0	6	94
Imipenem	0.25–4	0.5	2	100	0	0
Meropenem	0.25–8	0.5	2	94	6	0
Moxifloxacin	0.5–8	2	8			
Clindamycin	0.5–>128	4	>128	38	6	56
Chloramphenicol	2–32	4	8	94	0	6
Metronidazole	0.25–1	0.5	1	100	0	0
Bacteroides vulgatus (12)						
Penicillin	≤0.03–>128	128	>128	0	0	100
Ampicillin	0.06–>128	128	>128	0	0	100
Piperacillin	1–>128	128	>128	25	25	50
Ticarcillin	≤0.03–>128	>128	>128	33	8	58
AMP-sulbactam	0.12–64	16	32	33	67	0
Piperacillin-TAZ	≤0.03–>128	8	32	92	8	0
Ticarcillin-CVA	≤0.03–>128	2	16	100	0	0
Cefoxitin	1–>128	8	>128	50	17	33
Cefmetazole	0.12–>128	64	>128	8	25	67
Cefoperazone	0.12–>128	128	>128	42	33	25
Imipenem	0.12–16	0.5	2	100	0	0
Meropenem	≤0.03–8	0.5	2	100	0	0
Moxifloxacin	0.06–32	1	4			
Clindamycin	0.06–>128	8	>128	50	0	50
Chloramphenicol	0.5–32	4	4	100	0	0
Metronidazole	0.12–16	1	2	100	0	0
B. fragilis group, other species (20)						
Penicillin	≤0.03–>128	128	>128	0	0	100
Ampicillin	0.06–>128	128	>128	0	0	100
Piperacillin	1–>128	128	>128	25	15	60
Ticarcillin	≤0.03–>128	>128	>128	20	10	70
AMP-sulbactam	0.12–64	16	32	45	20	35
Piperacillin-TAZ	≤0.03–>128	8	32	95	5	0
Ticarcillin-CVA	≤0.03–>128	2	16	95	0	5
Cefoxitin	1–>128	32	>128	35	25	40
Cefmetazole	0.12–>128	64	>128	25	0	75
Cefoperazone	0.12–>128	128	>128	15	10	75
Imipenem	0.12–16	0.5	2	95	5	0
Meropenem	≤0.03–8	0.5	2	95	5	0
Moxifloxacin	0.06–32	1	4			
Clindamycin	0.06–>128	8	>128	35	5	60
Chloramphenicol	0.5–32	4	4	100	0	0
Metronidazole	0.12–16	1	2	100	0	0
Fusobacterium species[e] (19)						
Penicillin	≤0.03–>128	0.5	>128	55	0	45
Ampicillin	≤0.03–>128	16	>128	25	5	70
Piperacillin	0.12–>128	16	128	63	21	16
Ticarcillin	0.06–>128	8	64	79	11	11
AMP-sulbactam	≤0.03–8	2	8	100	0	0
Piperacillin-TAZ	≤0.03–64	4	16	95	5	0
Ticarcillin-CVA	0.5–32	4	32	100	0	0
Cefoxitin	0.25–16	4	16	100	0	0
Cefmetazole	≤0.03–32	4	32	95	5	0
Cefoperazone	≤0.03–64	8	64	68	5	26
Imipenem	0.12–1	0.25	1	100	0	0
Meropenem	≤0.03–8	0.12	5	95	5	0
Moxifloxacin	0.06–4	0.25	4			
Clindamycin	0.25–>128	4	>128	45	20	35
Chloramphenicol	0.5–32	2	8	95	0	5
Metronidazole	0.06–>128	0.5	>128	75	0	25
Prevotella species[f] (16)						
Penicillin	≤0.03–128	4	32	38	0	62
Ampicillin	≤0.03–>128	64	>128	31	6	63
Piperacillin	0.25–128	64	64	31	56	13
Ticarcillin	0.06–64	16	32	88	12	0
AMP-sulbactam	0.06–>128	4	16	88	12	0
Piperacillin-TAZ	≤0.03–64	0.06	64	75	25	0
Ticarcillin-CVA	0.06–>128	0.5	32	94	0	6
Cefoxitin	0.5–>128	8	32	88	6	6
Cefmetazole	0.12–>128	2	64	88	0	12
Cefoperazone	0.5–>128	128	128	13	19	68
Imipenem	0.06–4	0.25	2	100	0	0
Meropenem	0.06–1	0.12	0.5	100	0	0
Moxifloxacin	0.06–4	0.5	2			

Continued on following page

TABLE 1—Continued

Bacterium (no. of isolates tested) and antimicrobial agent	MIC (μg/ml)			%[a]		
	Range	50%	90%	S	I	R
Clindamycin	≤0.03–>128	0.25	>128	69	0	31
Chloramphenicol	1–4	2	4	100	0	0
Metronidazole	0.12–>128	2	32	81	6	13
Veillonella species[g] (20)						
Penicillin	≤0.03–16	2	16	25	5	70
Ampicillin	0.25–128	4	16	25	5	70
Piperacillin	≤0.03–128	16	64	85	10	5
Ticarcillin	0.06–128	32	32	90	5	5
AMP-sulbactam	0.12–4	2	4	100	0	0
Piperacillin-TAZ	0.12–>128	16	128	85	10	5
Ticarcillin-CVA	≤0.03–>128	32	128	90	5	5
Cefoxitin	0.06–128	16	32	65	25	10
Cefmetazole	≤0.03–32	1	8	95	5	0
Cefoperazone	0.06–128	16	64	60	20	20
Imipenem	≤0.03–4	0.5	2	100	0	0
Meropenem	≤0.03–0.5	0.12	0.5	100	0	0
Moxifloxacin	0.06–2	0.12	1			
Clindamycin	≤0.03–>128	8	>128	40	5	55
Chloramphenicol	0.5–8	1	4	100	0	0
Metronidazole	0.12–>128	4	>128	75	5	20
Peptostreptococcus species[h] (31)						
Penicillin	≤0.03–128	0.25	8	84	0	16
Ampicillin	≤0.03–128	0.5	64	87	0	13
Piperacillin	≤0.03–64	0.12	16	97	3	0
Ticarcillin	0.06–128	0.5	2	87	3	10
AMP-sulbactam	≤0.03–32	0.12	0.5	90	0	10
Piperacillin-TAZ	≤0.03–32	0.06	8	100	0	0
Ticarcillin-CVA	≤0.03–128	1	64	84	16	0
Cefoxitin	0.06–>128	2	32	87	10	3
Cefmetazole	≤0.03–128	2	32	81	16	3
Cefoperazone	≤0.03–>128	1	128	84	3	13
Imipenem	≤0.03–32	0.12	4	94	3	3
Meropenem	≤0.03–4	0.12	2	100	0	0
Moxifloxacin	≤0.03–32	0.25	4			
Clindamycin	0.06–>128	8	128	45	0	55
Chloramphenicol	1–32	2	8	94	3	3
Metronidazole	0.06–>128	1	>128	68	0	32
C. perfringens (20)						
Penicillin	≤0.03–0.12	≤0.03	0.12	100	0	0
Ampicillin	≤0.03–0.12	≤0.03	0.12	100	0	0
Piperacillin	≤0.03–≤0.03	≤0.03	≤0.03	100	0	0
Ticarcillin	0.06–0.5	0.25	0.5	100	0	0
AMP-sulbactam	≤0.03–0.12	≤0.03	0.12	100	0	0
Piperacillin-TAZ	≤0.03–0.25	0.06	0.12	100	0	0
Ticarcillin-CVA	0.12–1	0.5	1	100	0	0
Cefoxitin	0.5–2	0.5	1	100	0	0
Cefmetazole	≤0.03–0.25	0.06	0.12	100	0	0
Cefoperazone	≤0.03–2	≤0.03	1	100	0	0
Imipenem	≤0.03–0.06	0.06	0.06	100	0	0
Meropenem	≤0.03–≤0.03	≤0.03	≤0.03	100	0	0
Moxifloxacin	0.12–0.25	0.25	0.25			
Clindamycin	0.06–2	1	2	100	0	0
Chloramphenicol	0.5–2	2	2	100	0	0
Metronidazole	0.5–2	1	2	100	0	0
Clostridium species[i] (16)						
Penicillin	≤0.03–32	0.25	16	88	0	12
Ampicillin	≤0.03–64	0.12	32	69	6	25
Piperacillin	≤0.03–2	0.5	2	100	0	0
Ticarcillin	0.25–128	1	4	88	0	12
AMP-sulbactam	≤0.03–16	0.25	16	88	12	0
Piperacillin-TAZ	≤0.03–8	1	4	100	0	0
Ticarcillin-CVA	≤0.03–128	1	128	88	12	0
Cefoxitin	0.06–16	2	16	100	0	0
Cefmetazole	0.06–32	2	32	88	12	0
Cefoperazone	0.06–8	0.5	8	100	0	0
Imipenem	≤0.03–1	0.06	0.5	100	0	0
Meropenem	≤0.03–1	0.12	1	100	0	0
Moxifloxacin	0.12–2	0.25	0.5			
Clindamycin	≤0.03–4	0.5	4	88	12	0
Chloramphenicol	0.5–4	2	4	100	0	0
Metronidazole	0.12–>128	1	>128	69	0	31

[a] S, susceptible; I, intermediate; R, resistant.
[b] AMP, ampicillin.
[c] TAZ, tazobactam.
[d] CVA, clavulanic acid.
[e] Fusobacterium species included F. mortiferum (3), F. necrophorum (3), F. nucleatum (6), F. varium (6), and an unidentified Fusobacterium sp. (1).
[f] Prevotella species included P. buccae (4), P. intermedia (3), P. melaninogenicus (2), P. oralis (2), and unidentified Prevotella spp. (5).
[g] Veillonella species included V. parvulla (8) and unidentified Veillonella spp. (12).
[h] Peptostreptococcus species included P. anaerobius (10), P. magnus (15), P. micros (3), and unidentified Peptostreptococcus spp. (3).
[i] Clostridium species included C. clostridioforme (4), C. ramosum (4), C. butyricum (3), C. septicum (3), and C. sporogenes (2).

tance to cefoxitin varied greatly with species and country. In our institution, the percentage of resistance to cefoxitin rose from 1% in 1991 to 4% for *B. fragilis* and from 10 to 70% for *B. thetaiotaomicron*. The rate of resistance to cefoxitin among the *B. fragilis* group was 12.8% in Spain (4), 11.3% in Canada (1997) (7), and 1.7 to 14.2% depending on the species in the United States (1995 and 1996) (14), 2.9 to 34.5% in Japan (1990 to 1992) (15), and 5% in South Africa (6). MICs for many isolates were 32 μg/ml, which was considered by the NCCLS as intermediate in susceptibility. *Fusobacterium* isolates remained susceptible to cefoxitin. The high rates of resistance to cefoxitin in non-*B. fragilis Bacteroides* species were unusual. The use of cefoxitin and cefmetazole has not increased in the past 10 years. Therefore the reason for the high incidence of resistance to cefoxitin is unclear.

For all species, high rates of susceptibility to imipenem and meropenem were observed. Resistance to meropenem was low (<1%). One isolate of *B. fragilis* and one isolate of *Peptostreptococcus* displayed intermediate susceptibility, and another *Peptostreptococcus* isolate displayed resistance, to imipenem. In general, The MIC_{90}s of imipenem and meropenem were similar except that some isolates showed discordant susceptibilities to imipenem and meropenem. For example, six isolates of *B. fragilis* (five intermediate and one resistant) were not susceptible to meropenem, but only one isolate was not susceptible to imipenem. Eight percent of *B. thetaiotaomicron* isolates were resistant to imipenem but susceptible to meropenem. Compared to the data of our previous report, the rate of resistance to imipenem for *B. fragilis* has not increased, but the MIC_{50} (from 0.12 to 0.5 μg/ml) and MIC_{90} (from 1 to 4 μg/ml) have increased slightly. Resistance to the carbapenems has been occasionally and infrequently recorded (13).

Clindamycin has long been considered the drug of choice for treatment of anaerobes. However, over the past 20 years, there

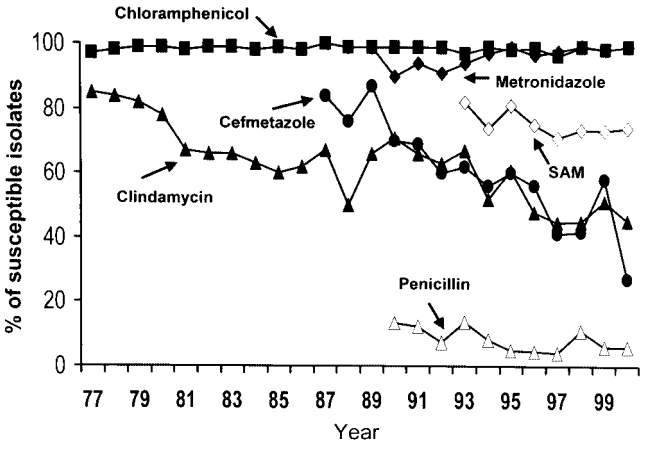

FIG. 1. Rates of susceptibility to six routinely tested agents in all of the *Bacteroides* species isolates at National Taiwan University Hospital from 1977 to 2000. Susceptibility testing was performed by the disk diffusion method before 1990 and by the breakpoint agar dilution method after 1991. SAM, ampicillin- sulbactam.

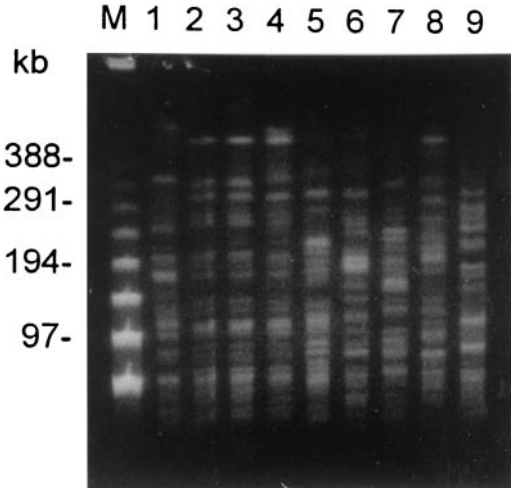

FIG. 2. PFGE analysis of *Xba*I-digested genomic DNA from *B. fragilis* isolates. Lane M, lambda ladder molecular size markers; lanes 1 to 6, metronidazole-nonsusceptible *B. fragilis* isolates (lanes 2 and 3, isolates from the same individual); lanes 7 to 9, metronidazole-susceptible *B. fragilis* isolates.

has been a significant increase in the rate of resistance to clindamycin among isolates of the *B. fragilis* group in many areas (1, 8, 11, 13–15). In our institution, the overall activities of clindamycin against the *B. fragilis* group were poor (33 to 70%). The high prevalence of resistance to clindamycin in *B. fragilis* group isolates has been described previously in several reports. For example, a high prevalence of resistance to clindamycin (49%) in the *B. fragilis* group was observed by Betriu et al. in Spain (4). In Korea, in 1994, the rates of resistance to clindamycin for *B. fragilis*, *B. thetaiotaomicron*, and other *Bacteroides* spp. were 38, 45.5, and 69%, respectively (8). However, in several areas the rate of resistance to clindamycin for the *B. fragilis* group remained low. In South Africa, only 5% of isolates were resistant to clindamycin in the *B. fragilis* group (6). It was also reported that clindamycin resistance is associated with hospital-acquired infections (11). Rates of resistance for isolates varied greatly with species and country. In the present study, high rates of resistance to clindamycin were found for the following organisms: *B. thetaiotaomicron* (70%), *B. caccae* (67%), *Bacteroides uniformis* (56%), *Veillonella* and *Peptostreptococcus* spp. (55%), *Fusobacterium* spp. (35%), and *B. fragilis* and *Prevotella* spp. (31%). *B. thetaiotaomicron* isolates were also more resistant to cefoxitin than other species. In agreement with other reports, the resistance rates for non-*B. fragilis* species of the *B. fragilis* group were found higher than that for *B. fragilis* (33%). Aldridge et al. reported that *Bacteroides distasonis* and *Bacteroides ovatus* were more resistant to clindamycin than other species (1). Since *B. thetaiotaomicron* is usually the second most frequently encountered *Bacteroides* species, rapid detection and identification are important. We recently described a PCR assay which provided a rapid and accurate method for identification of *B. thetaiotaomicron* (17).

Among gram-positive anaerobes, *C. perfringens* was the most susceptible. Other *Clostridium* species were less susceptible to penicillin, ampicillin, ticarcillin, and metronidazole. This result is similar to the finding of a study done in Korea (8) but is different from that of a study done in South Africa (6).

In the present study, the MIC results for moxifloxacin confirm the broad spectrum of its activity against gram-positive and gram-negative anaerobic bacteria. Although no interpretation standard is available for anaerobes, for the anaerobic bacteria tested, the moxifloxacin MIC_{50} varied from 0.12 to 2 μg/ml and MIC_{90} varied from 0.25 to 8 μg/ml. Many new fluoroquinolones have been tested for in vitro activities against gram-positive, gram-negative, and anaerobic bacteria previously (4, 9). Similarly, other studies have demonstrated the good activity of new fluoroquinolones against various anaerobic species. Moxifloxacin is, therefore, a potentially useful antibiotic against anaerobes.

The rates of resistance to metronidazole for several gram-positive anaerobes, *Peptostreptococcus* (32%) and *Clostridium* species (31%) other than *C. perfringens*, were higher than those for gram-negative anaerobes *Fusobacterium* (25%), *Veillonella* (20%), *Prevotella* (13%), and *Bacteroides* species (<3%). Previous reports also showed that peptostreptococci are generally less susceptible to metronidazole than gram-negative anaerobes. A similar percentage of resistance to metronidazole for peptostreptococci in Korea was described by Lee et al. (8). Resistance to metronidazole among *B. fragilis* isolates in Taiwan is first documented in this report. By PFGE analysis, five strains (four patterns) showed genotypic heterogeneity, suggesting that they correspond to a heterogeneous population rather than to the dissemination of a single clone. The results suggest that the emergence of these resistant strains may be sporadic. The development of antibiotic resistance in anaerobic bacteria has a tremendous impact on the selection of antimicrobial agents for empirical therapy. It suggests the need to monitor antibiotic susceptibility patterns of anaerobes related to geographic regions periodically.

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