대한진단검사의학회지 제29권 제4호 2009
Korean J Lab Med 2009;29:293-8
DOI 10.3343/kjlm.2009.29.4.293

국내 분리 *Bacteroides fragilis*군의 8년간 내성률 변화(1997-2004)

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Resistance Trends of *Bacteroides fragilis* Group Over an 8-Year Period, 1997-2004, in Korea

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Background : *Bacteroides fragilis* group organisms are the most frequently isolated anaerobes in human infections. Increasing resistance to various antimicrobial agents is a significant problem in choosing appropriate antimicrobial agents to treat anaerobic infections. Periodic monitoring of the regional resistance trends of *B. fragilis* group isolates is needed.

Methods : A total of 466 nonduplicate clinical isolates of *B. fragilis* group organisms (276 *B. fragilis*, 106 *Bacteroides thetaiotaomicron*, and 84 other *B. fragilis* group organisms) were collected during the 8-yr period from 1997 to 2004 in a Korean university hospital. Minimum inhibitory concentrations to various antimicrobial agents were determined by the CLSI agar dilution method.

Results : Eight isolates were resistant to imipenem. Additionally, the resistance rates to cefotetan were decreased in *B. thetaiotaomicron*, while those for clindamycin were significantly increased compared to the rates found in previous studies. Depending on species, resistance rates were 1-4% for imipenem, 1-6% for piperacillin-tazobactam, 4-11% for cefoxitin, 33-49% for piperacillin, 14-60% for cefotetan, and 51-76% for clindamycin. No isolates were resistant to chloramphenicol or metronidazole.

Conclusions : Piperacillin-tazobactam, cefoxitin, imipenem, chloramphenicol, and metronidazole are still active against *B. fragilis* group isolates, while clindamycin no longer has a value as an empirical therapeutic agent in Korea. Furthermore, this study identified the first imipenem-resistant *B. fragilis* group isolates in Korea. *(Korean J Lab Med 2009; 29:293-8)*

Key Words : *Bacteroides fragilis group, Antimicrobial susceptibility, Trend, Korea*

Received : February 20, 2009 Manuscript No : KJLM09-034
Revision received : May 8, 2009
Accepted : May 21, 2009
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*This work was supported by the Brain Korea 21 Project in 2005.

INTRODUCTION

Bacteroides fragilis group organisms are not only the most frequently isolated anaerobes in human infections, but also the most common cause of anaerobic bacteremia with a relatively high rate of mortality [1-4]. *B. fragilis* group includes *B. fragilis*, *B. thetaiotaomicron*, *B. vulga-*

tus, *B. ovatus*, *B. distasonis*, *B. uniformis*, *B. caccae*, *B. eggerthii*, *B. merdae*, and *B. stercori*. Increasing resistance to antimicrobial agents is a significant problem among anaerobic bacteria [5, 6]. Clinical outcome of *B. fragilis* group infection is also affected by appropriate antimicrobial agent treatment [7]. In our previous studies, *B. fragilis* group organisms in Korea were shown to be more frequently resistant to various antimicrobial agents than in other countries [8, 9]. The CLSI does not recommend routine susceptibility testing for anaerobic microbes [10]. Instead, periodic monitoring of the regional resistance trends of clinically important anaerobes, including *B. fragilis* group isolates, need to be performed to assist in the selection of empirical antimicrobial agents to treat these anaerobic infections [11]. However, there have been no recent studies on susceptibility testing against *B. fragilis* group isolates from Korea. Therefore, providing current susceptibility patterns of these isolates is very important for appropriate empirical antimicrobial therapy.

In this study, we attempted to determine the current susceptibilities of *B. fragilis* group organisms isolated from patients in Korea during a 8-yr peoriod (1997-2004).

MATERIALS AND METHODS

1. Isolates

All *B. fragilis* group organisms were isolated between 1997 and 2004 from various clinical specimens (blood, body fluid, and pus) in a university hospital in Korea. The specimens were inoculated on phenyl ethyl alcohol blood agar plates and in thioglycolate broth, which were then incubated at 37℃ for 48 hr in an anaerobic chamber (Forma, Marietta, OH, USA) with an atmosphere of 10% H_2, 10% CO_2, and 80% N_2. Gram stain was performed as preliminary examination. The isolates were identified by conventional methods, ANI card (bioMerieux, Marcy l'Etoile, France, and/or the ATB 32A system (bioMerieux, Marcy l'Etoile, France) [12, 13]. The isolates were stored at −70℃ in 20% skim milk (BBL Microbiology Systems, Cockeysville, MD, USA) until used for the study.

2. Antimicrobial susceptibility

Antimicrobial susceptibility was tested by the CLSI agar dilution method [10]. An inoculum of 10^5 CFU/spot was applied with a Steers replicator (Craft Machine, Chester, PA, USA) onto the surface of the brucella agar plates supplemented with vitamin K_1 (10 μg/mL) and 5% laked sheep blood. The plates were incubated at 37℃ for 48 hr in an anaerobic chamber with an atmosphere of 10% H_2, 10% CO_2, and 80% N_2.

Antimicrobial agents used were as follows: piperacillin and tazobactam (Yuhan, Seoul, Korea), cefoxitin (Merck Sharp & Dohme, West Point, PA), cefotetan (Daiichi Pharmaceutical, Tokyo, Japan), clindamycin (Korea Upjohn, Seoul, Korea), imipenem and metronidazole (Choong Wae, Seoul, Korea), and chloramphenicol (Chong Kun Dang, Seoul, Korea). For the combination of piperacillin and tazobactam, a constant amount of tazobactam (4 μg/mL, final concentration) was added to piperacillin. An anaerobic chamber was used for anaerobic incubation, and American Type Culture Collection (ATCC) strains of *B. fragilis* 25285 and *B. thetaiotaomicron* ATCC 29741 were used as controls. The breakpoints recommended by CLSI for anaerobic bacteria were applied to interpret the MICs [10].

RESULTS

A total of 466 nonduplicate clinical isolates of *B. fragilis* groups organisms, which were isolated during an 8-yr period, were tested for their susceptibility to antibiotics (Table 1). *B. fragilis* was the most common species within *Bacteroides* spp. (30-42 isolates per yr, 59.2%), followed by *B. thetaiotaomicron* (9-22 isolates per yr, 22.7%). Other *Bacteroides* spp. included *B. vulgates* (30 isolates), *B. ovatus* (27 isolates), *B. distasonis* (25 isolates), *B. uniformis* (I isolate), and *B. caccae* (1 isolate). There were no remarkable differences in the distribution of *Bacteroides* spp. isolated during the collection period.

MIC ranges, MIC_{50}s, MIC_{90}s, and the percentages of resistant isolates for various antimicrobial agents are shown in Table 2. The most active β-lactam agent was imipen-

Table 1. Distribution of the species within the *Bacteroides fragilis* group organisms isolated from 1997 to 2004

Bacteroides species	N (%) isolates tested								
	1997	1998	1999	2000	2001	2002	2003	2004	Total
B. fragilis	34	30	30	40	34	30	36	42	276 (59.2)
B. thetaiotaomicron	9	11	10	12	15	22	16	11	106 (22.7)
B. vulgatus	5	4	7	1	4	4	3	2	30 (6.4)
B. ovatus	6	4	7	4	2	1	1	2	27 (5.8)
B. distasonis	3	3	6	3	4	3	2	1	25 (5.4)
B. uniformis	0	0	0	0	1	0	0	0	1 (0.2)
B. caccae	0	0	0	0	0	0	1	0	1 (0.2)
Total	57	52	60	60	60	60	59	58	466 (100.0)

em, followed by piperacillin-tazobactam. The imipenem resistance in *B. fragilis*, *B. thetaiotaomicron*, and other *Bacteroides* spp. were 1%, 2%, and 4%, respectively. We found eight imipenem-resistant isolates; three *B. fragilis*, two *B. thetaiotaomicron*, and three *B. distasonis* isolates were inhibited by ≥16 μg/mL of imipenem. MIC_{50}s and MIC_{90}s of piperacillin-tazobactam were 0.5 and 4 μg/mL, respectively, for *B. fragilis*, 8 and 16 μg/mL for *B. thetaiotaomicron*, and 4 and 16 μg/mL for other *Bacteroides* spp. Cefoxitin was the third most active β-lactam drug with resistance rates of 4, 6, and 11% for *B. fragilis*, *B. thetaiotaomicron*, and other *Bacteroides* spp, respectively. Piperacillin and cefotetan were less active and most strains tested (90%) were inhibited by these drugs at >256 and ≥128 μg/mL, respectively. The resistance rates to clindamycin were 51% in *B. fragilis*, 76% in *B. thetaiotaomicron*, and 74% in other *Bacteroides* spp. All the strains were inhibited by ≤8 μg/mL of chloramphenicol or metronidazole, to which no isolates were resistant. The resistance rates to various antimicrobial agents of the non-*fragilis* species were higher than those of *B. fragilis* (Table 2).

The trends from 1997 to 2004 of the resistance rates of *B. fragilis* group organisms to five antimicrobial agents are shown in Fig. 1. The rates of resistance to piperacillin for *B. thetaiotaomicron* varied with the highest resistance rate reaching 63% in 2003. Also, the resistance rate to piperacillin for other *Bacteroides* spp. varied from 36% in 1997 to 60% in 2004. The rates of resistance to piperacillin-tazobactam and cefoxitin remained low in the

Table 2. Antimicrobial susceptibility of *Bacteroides fragilis* group organisms isolated from 1997 to 2004

Organism (N isolates) and antimicrobial agents	MIC range	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	% Resistant
Bacteroides fragilis (276)				
Piperacillin	2->256	16	>256	33
Piperacillin-tazobactam	0.03->128	0.5	4	1
Cefoxitin	4->128	8	32	4
Cefotetan	2->128	8	128	14
Imipenem	0.06-128	0.25	0.5	1
Clindamycin	≤0.06->128	8	>128	51
Chloramphenicol	2-8	4	8	0
Metronidazole	0.5-8	4	8	0
Bacteroides thetaiotaomicron (106)				
Piperacillin	4->256	64	>256	42
Piperacillin-tazobactam	0.12->256	8	16	4
Cefoxitin	4->128	16	32	6
Cefotetan	8->128	64	>128	60
Imipenem	0.03-32	0.5	2	2
Clindamycin	1->128	>128	>128	76
Chloramphenicol	2-8	8	8	0
Metronidazole	1-8	2	4	0
Other *Bacteroides* spp. (84)				
Piperacillin	4->256	64	>256	49
Piperacillin-tazobactam	0.03->256	4	16	6
Cefoxitin	2->128	16	64	11
Cefotetan	2->128	64	>128	54
Imipenem	0.03-32	0.5	1	4
Clindamycin	0.06->128	>128	>128	74
Chloramphenicol	2-8	4	8	0
Metronidazole	0.5-8	2	4	0

range of 0% to 9% for *B. fragilis*, 0% to 17% for *B. thetaiotaomicron*, and 0% to 27% for other *Bacteroides* spp. The resistance rate of *B. fragilis* against cefotetan increased gradually from 15% in 1997 to 29% in 2004. Interestingly, the resistance rate of *B. thetaiotaomicron* against cefotetan decreased from 100% in 1997 to 27% in 2004. In contrast, the rate of clindamycin resistance for *B. thetaiotaomicron* increased gradually from 67% in 1997 to 91% in 2004.

DISCUSSION

In this study, the susceptibilities of 466 isolates of *B. fragilis* group organisms to various antimicrobial agents were determined. Among β-lactam agents, imipenem was the most active, but three *B. fragilis*, two *B. thetaiotaomicron* and three *B. distasonis* isolates were inhibited by

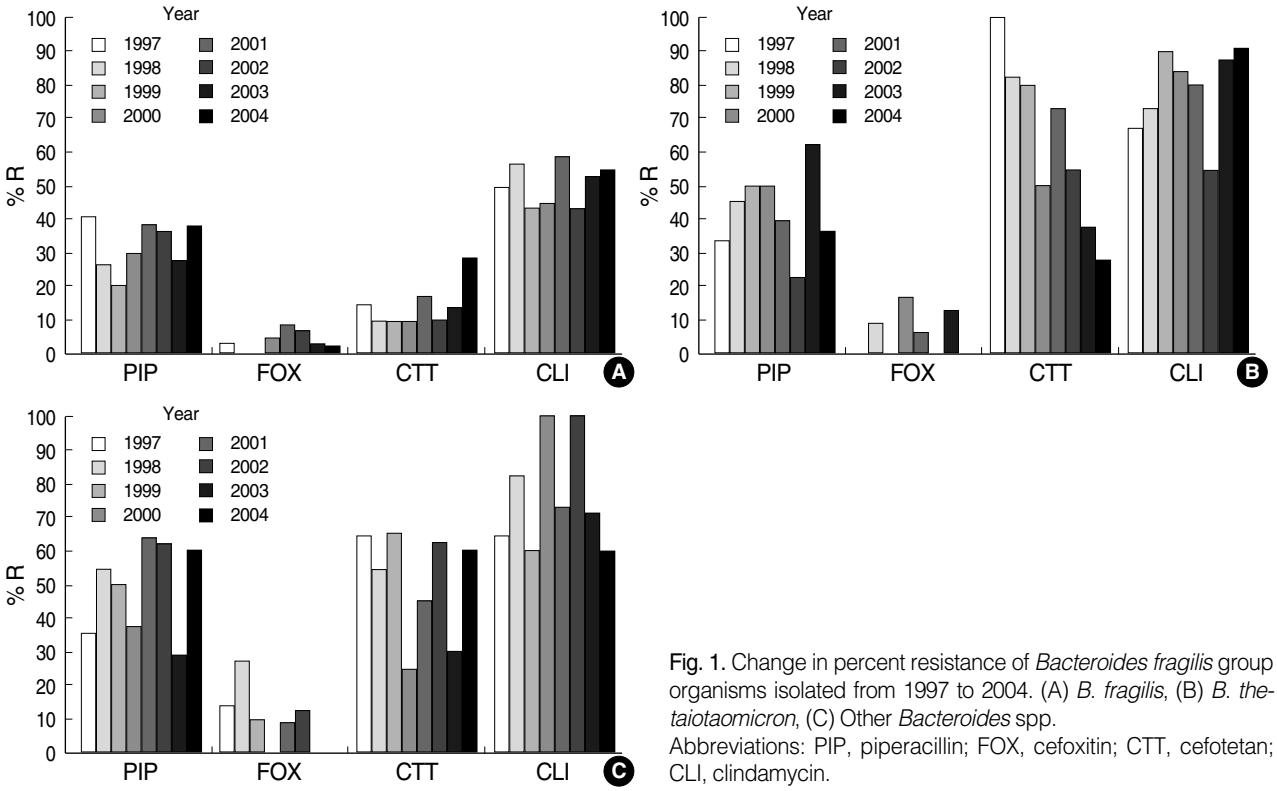

Fig. 1. Change in percent resistance of *Bacteroides fragilis* group organisms isolated from 1997 to 2004. (A) *B. fragilis*, (B) *B. thetaiotaomicron*, (C) Other *Bacteroides* spp.
Abbreviations: PIP, piperacillin; FOX, cefoxitin; CTT, cefotetan; CLI, clindamycin.

≥16 μg/mL of imipenem. Even though imipenem is one of the strongest β-lactamase inducers, it is recommended for the treatment of anaerobic infections, especially intra-abdominal infections, because carbapenems are highly active against polymicrobial infection including *B. fragilis* group organisms and other *Enterobacteriacea* [14]. The imipenem resistance rate of *B. fragilis* group isolates in this study (1.9%) was similar to rates of 0.6-1.3% seen in Europe, 0.4% in Spain, and 0.5% in the USA [15-17]. A well-known mechanism of carbapenem resistance in *B. fragilis* group is *cfiA* and insertion sequence (IS) elements [18]. Presenting *cfiA* and IS elements simultaneously, can reduce susceptibility to carbapenems [19, 20].

Most *B. fragilis* group organisms are naturally resistant to many penicillins and cephalosporins because of their production of chromosomal class A β-lactamases with predominantly cephalosporinase activity [21]. However, the combination of these drugs with β-lactamase inhibitors restores the antimicrobial activity of β-lactams. In this study, the resistance rates for piperacillin were 33-49% (Table 2), an increase of approximately 10% in comparison with previous Korean studies [9]. However, the resistance rates to piperacillin-tazobactam were ≤1% for *B. fragilis* and 4-6% for non-*fragilis* species. Piperacillin-tazobactam was more active than piperacillin alone against *B. fragilis* groups in vitro and other in vivo study [22].

Cefoxitin and cefotetan are cephamycin group antimicrobial agents that are commonly used for aerobic and anaerobic infections. However, the resistance rates to both of the cephamycins were significantly different depending on the species of bacteria tested. The resistance rates to cefoxitin, the third most active β-lactam in this study, were 4-11% overall for *B. fragilis* group isolates, and these rates were not significantly higher as compared with previous studies, especially the rates for *B. fragilis* [9, 23]. However, the resistance rates to cefotetan (14-60%) were significantly higher than those to cefoxitin. It is interesting that trends of cefotetan resistance in *B. thetaiotaomicron* decreased from 100% in 1997 to 27% in 2004. This apparent change might be due to the small number of the isolates (9-22 per year). Further surveillance, including more isolates collected after 2004, will be required to eval-

uate this trend. In contrast, the resistance rate of *B. fragilis* to cefotetan increased from 15% in 1997 to 29% in 2004, a nearly two-fold increase.

Compared with the previous studies done in Korea, the clindamycin resistance rate significantly increased from 18% in 1989-1990 to 51% in 1997-2004 for *B. fragilis*, and from 32-38% in 1989-1990 to 74-76% in 1997-2004 for non-*fragilis* species [9]. These results indicate resistance rates that are more than 20% higher than those found in a survey done in the United States (20-25% in 2001-2004) [6], but are similar with susceptibility data collected in Taiwan (55-65% in 2001-2004) [24]. Clindamycin resistance of anaerobes is due to an alteration in ribosome as the target site, which is similar to the cause of macrolide-lincosamide-streptogramin resistance in staphylococci [25].

Chloramphenicol and metronidazole are still the most active non-β-lactam agents against anaerobic bacteria. None of the isolates were found to be resistant to these drugs (≤ 8 μg/mL) in this study, although one strain resistant to metronidazole has been reported in the U.S. [6, 26-28].

In conclusion, this study provides insights into the current resistance trends of various species of the *B. fragilis* group. Among β-lactam agents, imipenem, piperacillin-tazobactam, and cefoxitin are still active against *B. fragilis* group organisms and they can be used for empirical therapy. Metronidazole and chloramphenicol retain excellent in vitro antimicrobial activities against this group of bacteria and they are still useful for clinical cases. Continuous investigation will be required to demonstrate continuing changes in susceptibility patterns of *B. fragilis* group isolates.

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