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Resistance Trends of the *Bacteroides fragilis* Group over a 10-Year Period, 1997 to 2006, in Madrid, Spain▽

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The changes in susceptibilities of *Bacteroides fragilis* group strains isolated in our hospital from 1997 to 2006 were studied. A total of 1,343 clinical strains were included. The study showed differences in the resistance rates in the different species of the group. Increasing resistance to clindamycin and moxifloxacin was observed. Susceptibility to imipenem, piperacillin-tazobactam, and metronidazole remained unchanged.

Members of the *Bacteroides fragilis* group can cause infections as serious as intra-abdominal infection, postoperative wound infection, and bacteremia. An increase in the mean incidence of anaerobic bacteremia has recently been noted by Lassmann et al. (9), with the most commonly isolated organisms being those of the *B. fragilis* group. Over the past 20 years, geographic variations and increasing resistance of this group to several of the traditionally used antimicrobial agents and some of the newer β-lactam agents have been reported (1, 2, 6, 14, 17, 18). Periodic monitoring of the susceptibility patterns of these organisms is now recommended. We studied the susceptibilities of *B. fragilis* group strains isolated in our hospital from 1997 to 2006 to determine changes and to detect resistance trends.

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The study included a total of 1,343 *B. fragilis* group clinical strains isolated in our hospital from 1997 to 2006. Only one isolate per patient was studied to avoid duplication. The isolates were recovered from the following sources: abdomen (45.6%), skin and soft tissue (40.1%), blood (10.3%), genital tract (2.1%), respiratory tract (0.9%), and other sites (0.9%). Species were identified using the Rapid ID 32A system (bioMérieux, Marcy l'Etoile, France). Reference strains *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were used as controls. The MICs obtained by testing the quality control strains in parallel with test strains were within the acceptable range indicated by CLSI (formerly the NCCLS) for each antimicrobial agent tested.

Susceptibilities were determined by the agar dilution method according to CLSI criteria (10). The following agents were studied: metronidazole, chloramphenicol, clindamycin, cefoxitin, imipenem, amoxicillin-clavulanate, piperacillin-tazobactam, moxifloxacin, and tigecycline (tested since 2000). To calculate antibiotic resistance rates, the CLSI-approved breakpoints (4) were used. The breakpoint for tigecycline resistance used was that established by the U.S. Food and Drug Administration.

The most frequent species isolated within the group was *B. fragilis* (62%), followed by *B. thetaiotaomicron* (13.5%), *B. uniformis* (7.8%), *B. vulgatus* (3.5%), *B. caccae* (3.3%), *B. distasonis* (3.2%), *B. ovatus* (3.1%), *B. eggerthii* (0.6%), *B. stercoris* (0.5%), *B. merdae* (0.5%), and *Bacteroides* spp. (1.8%). There were no significant differences in the distribution of the various species during the study period.

MIC ranges, MICs at which 50% and 90% of bacteria were inhibited (MIC_{50}s and MIC_{90}s), and the percentage of resistant strains for each antimicrobial agent are summarized in Table 1. We did not observe resistance to metronidazole, although several authors have detected resistance to this agent (6, 8, 14, 16, 19). Chloramphenicol MICs were near the susceptibility breakpoint; 58.5% of the isolates had an MIC of 4 μg/ml and 25.5% had an MIC of 8 μg/ml. We found two isolates (one *B. distasonis* isolate and one *B. merdae* isolate) with intermediate resistance to chloramphenicol. Consistent with the work of other authors (1, 17), our study shows variability in the resistance patterns of the different species of the group. *B. fragilis* isolates were more susceptible to cefoxitin, amoxicillin-clavulanate, clindamycin, moxifloxacin, and tigecycline than were the other species of the group. Overall resistance to clindamycin was 38.3%, and this was higher among *B. ovatus* and *B. caccae* (48.8% and 46.7%, respectively) and lower among *B. fragilis* (33%) strains. Most strains tested (81.4%) were inhibited by tigecycline at ≤4 μg/ml. This activity is consistent with that reported by other groups (7, 8, 16). *B. vulgatus* and *B. fragilis* were the species most susceptible to tigecycline (more than 85% of the isolates were inhibited at 4 μg/ml), and *B. caccae* was the least susceptible (74.2% of the isolates inhibited at 4 μg/ml).

The MIC_{50}s and MIC_{90}s of moxifloxacin for *B. fragilis* (0.5/4 μg/ml) were four and eight dilutions, respectively, lower than those for *B. uniformis*, *B. caccae*, and *B. ovatus* (2/32 μg/ml). The overall rate of resistance to moxifloxacin was 13.9%, ranging from 9.7% for *B. fragilis* to 24.5% for *B. uniformis*. However, Snydman at al. (16) reported higher rates of moxifloxacin resistance for the different species of the group, ranging from 27.3% for *B. fragilis* to 54.7% for *B. vulgatus*. The rate of cefoxitin resistance, around 10%, is similar to that recently

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TABLE 1. In vitro activities of several antimicrobial agents against 1,343 *B. fragilis* group isolates (1997 to 2006)

Organism (n^a) and antimicrobial agent	MIC (μg/ml)			% Resistant strains[b]
	Range	50%	90%	
B. fragilis group (1,343)				
Metronidazole	≤0.06–8	1	2	0
Chloramphenicol	0.125–16	4	8	0
Clindamycin	≤0.06–>256	2	>256	38.3
Tigecycline	≤0.06–32	1	8	7.4
Moxifloxacin	≤0.06–128	1	8	13.9
Amoxicillin-clavulanate	≤0.06–256	1	8	10.1
Piperacillin-tazobactam	≤0.06–256	2	16	0.8
Cefoxitin	≤0.06–256	16	32	10.8
Imipenem	≤0.06–>256	0.25	1	0.4
B. fragilis (833)				
Metronidazole	≤0.06–8	1	2	0
Chloramphenicol	0.125–8	4	8	0
Clindamycin	≤0.06–>256	2	>256	33.3
Tigecycline	≤0.06–16	1	8	6.5
Moxifloxacin	≤0.06–64	0.5	4	9.7
Amoxicillin-clavulanate	≤0.06–256	1	8	6.2
Piperacillin-tazobactam	≤0.06–>256	1	8	0.5
Cefoxitin	≤0.06–256	8	32	6.4
Imipenem	≤0.06–>256	0.25	1	0.6
B. thetaiotaomicron (182)				
Metronidazole	≤0.06–4	1	2	0
Chloramphenicol	0.125–8	4	8	0
Clindamycin	≤0.06–>256	4	>256	47.3
Tigecycline	≤0.06–16	1	8	7.5
Moxifloxacin	≤0.06–64	2	16	17
Amoxicillin-clavulanate	0.125–64	2	16	4.9
Piperacillin-tazobactam	≤0.06–>256	16	32	3.3
Cefoxitin	≤0.06–256	32	64	23.6
Imipenem	≤0.06–32	0.5	2	0.5
B. uniformis (106)				
Metronidazole	0.125–4	1	2	0
Chloramphenicol	1–8	4	8	0
Clindamycin	≤0.06–>256	2	>256	40.6
Tigecycline	≤0.06–16	1	8	9.1
Moxifloxacin	≤0.06–128	2	32	24.5
Amoxicillin-clavulanate	0.25–64	1	16	12.3
Piperacillin-tazobactam	≤0.06–32	4	16	0
Cefoxitin	≤0.06–128	16	32	9.4
Imipenem	≤0.06–4	0.25	2	0
B. vulgatus (47)				
Metronidazole	0.25–4	1	2	0
Chloramphenicol	1–8	4	8	0
Clindamycin	≤0.06–>256	4	>256	44.7
Tigecycline	0.125–16	0.5	8	6.1
Moxifloxacin	0.5–64	2	16	17
Amoxicillin-clavulanate	0.25–32	2	16	21.3
Piperacillin-tazobactam	≤0.06–64	8	32	0
Cefoxitin	1–256	16	64	17
Imipenem	≤0.06–4	0.5	2	0
B. caccae (45)				
Metronidazole	0.125–8	1	2	0
Chloramphenicol	1–8	4	8	0
Clindamycin	≤0.06–>256	4	>256	46.7
Tigecycline	0.125–64	1	16	16.1
Moxifloxacin	0.25–64	2	32	22.2
Amoxicillin-clavulanate	0.125–32	2	16	20
Piperacillin-tazobactam	≤0.06–128	8	32	2.2
Cefoxitin	≤0.06–64	16	32	8.9
Imipenem	≤0.06–4	0.5	2	0

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TABLE 1—*Continued*

Organism (n^a) and antimicrobial agent	MIC (μg/ml)			% Resistant strains[b]
	Range	50%	90%	
B. distasonis (43)				
Metronidazole	0.5–4	1	2	0
Chloramphenicol	1–16	8	8	0
Clindamycin	≤0.06–>256	4	>256	41.9
Tigecycline	0.125–32	2	8	8.3
Moxifloxacin	≤0.06–128	1	16	18.6
Amoxicillin-clavulanate	0.125–64	4	16	16.3
Piperacillin-tazobactam	≤0.06–32	16	16	0
Cefoxitin	≤0.06–128	32	64	25.6
Imipenem	≤0.06–4	1	1	0
B. ovatus (41)				
Metronidazole	0.125–4	1	2	0
Chloramphenicol	0.25–8	4	8	0
Clindamycin	≤0.06–>256	4	>256	48.8
Tigecycline	0.125–16	2	8	7.2
Moxifloxacin	0.125–32	2	32	24.4
Amoxicillin-clavulanate	0.125–32	1	16	17.1
Piperacillin-tazobactam	≤0.06–32	8	16	0
Cefoxitin	0.5–128	16	64	12.2
Imipenem	≤0.06–8	0.25	2	0
Other *B. fragilis* group species (46)[c]				
Metronidazole	0.125–4	0.5	2	0
Chloramphenicol	2–16	4	8	0
Clindamycin	≤0.06–>256	4	>256	45.7
Tigecycline	0.125–16	1	8	10.5
Moxifloxacin	0.25–128	2	16	28.3
Amoxicillin-clavulanate	0.25–64	2	16	19.6
Piperacillin-tazobactam	≤0.06–32	8	32	0
Cefoxitin	2–128	16	64	23.9
Imipenem	≤0.06–4	0.25	2	0

[a] *n*, no. of isolates.
[b] MICs for resistant isolates are those described by CLSI. The breakpoint for tigecycline is that recommended by the FDA.
[c] *Bacteroides* spp., 24 isolates; *B. eggerthii*, 8 isolates; *B. merdae*, 7 isolates; and *B. stercoris*, 7 isolates.

described by Snydman et al. (16). *B. distasonis* and *B. thetaiotaomicron* were the species that exhibited the highest level of resistance to cefoxitin (25.6% and 23.6% of resistance, respectively) and *B. fragilis* the lowest level (6.4% of resistance). Imipenem was the most active β-lactam agent tested, followed by piperacillin-tazobactam. Imipenem resistance was detected in only five isolates (four *B. fragilis* isolates and one *B. thetaiotaomicron* isolate), four of which were highly resistant to imipenem and to all other β-lactam agents tested. *B. fragilis* was more susceptible than *B. thetaiotaomicron* to piperacillin-tazobactam (0.5% of resistance versus 3.3%).

The evolution of antibiotic resistance among *B. fragilis* group strains isolated in our hospital from 1997 to 2006 is shown in Table 2. Rates of resistance to clindamycin remained stable in the 33% to 35% range until 1998 and increased to 42.5% in 1999. In 2006, we found a clindamycin resistance rate of 47.9%, higher than the rates recently detected by other groups, that is, between 23% and 39% (8, 13, 16, 19). Resistance to cefoxitin decreased from 12.8% in 1997 to 3.4% in 1999 ($P < 0.04$); this rate increased to 27% in 2006 ($P < 0.0001$). The decreased activity of cefoxitin noted during the latter part of our study has also been reported in Belgium by Wybo et al. (19). The MIC_{50}s and MIC_{90}s for tigecycline did not change over the 7 years of testing. Because tigecycline has been available in Spain since October 2006, it would be interesting to perform periodic susceptibility studies to assess the evolution of the susceptibility patterns over time. No change in the susceptibilities to metronidazole and chloramphenicol was observed.

As we previously reported (3), there is a continuing trend toward higher MIC_{50}s and MIC_{90}s for moxifloxacin. The percentage of strains inhibited by moxifloxacin at ≥8 μg/ml increased from 6% in 1997 to 25% in 2006. The MIC_{90} for moxifloxacin increased eightfold during the same period (from 2 μg/ml to 16 μg/ml). This trend of increased resistance to moxifloxacin has also been described by other authors in Spain (12) and elsewhere (5, 17). More recently, in the United States, Snydman et al. (16) reported significant increases in the rates of resistance to moxifloxacin during the period 1997 to 2004 for most species of the *B. fragilis* group.

A slight increase in the rate of resistance to amoxicillin-clavulanate was observed during the last 7 years of the study. This fact could be associated with the increased use of this agent in our area. Imipenem-resistant *B. fragilis* group strains were isolated for the first time in our laboratory in 1989 (2). Since then, the incidence of such resistance has remained low and did not change appreciably between 1997 and 2006, ranging from 0% to 1.5%. By contrast with Snydman et al. (16), we did not detect a significant trend of lowered MICs for imi-

TABLE 2. Change in susceptibility patterns of *B. fragilis* group from 1997 to 2006

Antimicrobial agent	Yr	No. of isolates	MIC (μg/ml)			% Resistant strains[a]
			Range	50%	90%	
Cefoxitin						
	1997	218	≤0.06–128	16	64	12.8
	1998	163	≤0.06–256	8	32	7.3
	1999	146	≤0.06–128	8	32	3.4
	2000	140	1–256	8	32	4.3
	2001–2002	260	1–128	8	32	6.9
	2003–2004	139	0.5–256	16	32	9.4
	2005	137	≤0.06–128	16	64	16.8
	2006	140	4–256	16	64	27.1
Clindamycin	1997	218	≤0.06–>256	0.5	>256	33.5
	1998	163	≤0.06–>256	2	>256	35.6
	1999	146	≤0.06–>256	2	>256	42.5
	2000	140	≤0.06–>256	1	>256	29.3
	2001–2002	260	≤0.06–>256	2	>256	39.2
	2003–2004	139	≤0.06–>256	2	>256	42.4
	2005	137	≤0.06–>256	2	>256	39.4
	2006	140	≤0.06–>256	4	>256	47.9
Moxifloxacin	1997	218	≤0.06–32	0.5	2	6
	1998	163	≤0.06–32	0.5	4	6.7
	1999	146	≤0.06–128	0.5	4	8.9
	2000	140	≤0.06–32	0.25	8	11.4
	2001–2002	260	0.125–128	1	8	16.5
	2003–2004	139	0.25–64	2	16	25.9
	2005	137	0.125–128	1	8	19
	2006	140	0.125–64	2	16	25
Amoxicillin-clavulanate	1997	218	≤0.06–64	0.5	8	5
	1998	163	0.5–64	2	16	7.9
	1999	146	0.5–64	1	16	8.9
	2000	140	0.125–256	2	16	10.7
	2001–2002	260	0.125–64	1	8	5.4
	2003–2004	139	0.125–64	2	16	20.9
	2005	137	≤0.06–16	0.5	4	2.2
	2006	140	0.25–64	1	16	17.9

[a] MICs for resistant isolates are those described by CLSI.

penem. Rates of resistance to piperacillin-tazobactam also remained unchanged over time. The main findings observed in this study—increasing resistance to clindamycin and no change in rates of resistance to imipenem, piperacillin-tazobactam, and metronidazole—are consistent with those published in the most recent surveillance studies (16, 19).

Several authors have highlighted the importance of an appropriate choice of therapy in the clinical outcome of anaerobic infections (11, 15). The changing pattern of susceptibility of *B. fragilis* group strains isolated in our hospital over the past 10 years emphasizes the need to monitor the antibiotic susceptibility patterns of *B. fragilis* group organisms in order to guide the selection of appropriate antimicrobial therapy.

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REFERENCES

1. **Aldridge, K. E., D. Ashcraft, K. Cambre, C. L. Pierson, S. G. Jenkins, and J. E. Rosenblatt.** 2001. Multicenter survey of the changing in vitro antimicrobial susceptibilities of clinical isolates of *Bacteroides fragilis* group, *Prevotella*, *Fusobacterium*, *Porphyromonas*, and *Peptostreptococcus* species. Antimicrob. Agents Chemother. **45:**1238–1243.
2. **Betriu, C., C. Cabronero, M. Gómez, and J. J. Picazo.** 1992. Changes in the susceptibility of *Bacteroides fragilis* group organisms to various antimicrobial agents 1979–1989. Eur. J. Clin. Microbiol. Infect. Dis. **11:**352–356.
3. **Betriu, C., I. Rodríguez-Avial, M. Gómez, E. Culebras, and J. J. Picazo.** 2005. Changing patterns of fluoroquinolone resistance among *Bacteroides fragilis* group organisms over a 6-year period (1997–2002). Diagn. Microbiol. Infect. Dis. **53:**221–223.
4. **Clinical Laboratory Standards Institute.** 2007. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard. CLSI document M11-A7. CLSI, Wayne, Pa.
5. **Golan, Y., L. A. McDermott, N. V. Jacobus, E. J. Goldstein, S. Finegold, L. J. Harrell, D. W. Hecht, S. G. Jenkins, C. Pierson, R. Venezia, J. Rihs, P. Iannini, S. L. Gorbach, and D. R. Snydman.** 2003. Emergence of fluoroquinolone resistance among *Bacteroides* species. J. Antimicrob. Chemother. **52:**208–213.
6. **Horn, R., and H. G. Robson.** 2001. Susceptibility of the *Bacteroides fragilis* group to newer quinolones and other standard anti-anaerobic agents. J. Antimicrob. Chemother. **48:**127–130.
7. **Jacobus, N. V., L. A. McDermott, R. Ruthazer, and D. R. Snydman.** 2004. In vitro activities of tigecycline against the *Bacteroides fragilis* group. Antimicrob. Agents Chemother. **48:**1034–1036.
8. **Katsandri, A., A. Avlamis, A. Pantazatou, G. L. Petrikkos, N. J. Legakis, and J. Papaparaskevas.** 2006. In vitro activities of tigecycline against recently isolated Gram-negative anaerobic bacteria in Greece, including metronidazole-resistant strains. Diagn. Microbiol. Infect. Dis. **55:**231–236.
9. **Lassmann, B., D. R. Gustafson, C. M. Wood, and J. E. Rosenblatt.** 2007. Reemergence of anaerobic bacteremia. Clin. Infect. Dis. **44:**895–900.
10. **National Committee for Clinical Laboratory Standards.** 2004. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 6th ed. Approved standard. NCCLS publication M11-A6. NCCLS, Wayne, Pa.
11. **Nguyen, M. H., V. L. Yu, A. J. Morris, L. McDermott, M. W. Wagener, L. Harrell, and D. R. Snydman.** 2000. Antimicrobial resistance and clinical outcome of *Bacteroides* bacteremia: findings of a multicenter prospective observational trial. Clin. Infect. Dis. **30:**870–876.

12. **Oteo-Iglesias, J., J. I. Alós, and J. L. Gómez-Garcés.** 2002. Increase in resistance to new fluoroquinolones from 1998 to 2001 in the *Bacteroides fragilis* group. J. Antimicrob. Chemother. **50:**1055–1057.
13. **Papaparaskevas, J., A. Pantazatou, A. Katsandri, N. J. Legakis, and A. Avlamis.** 2005. Multicentre survey of the in-vitro activity of seven antimicrobial agents, including ertapenem, against recently isolated Gram-negative anaerobic bacteria in Greece. Clin. Microbiol. Infect. **11:**820–824.
14. **Rotimi, V. O., M. Khoursheed, J. S. Brazier, W. Y. Jamal, and F. B. Khodakhast.** 1999. *Bacteroides* species highly resistant to metronidazole: an emerging clinical problem? Clin. Microbiol. Infect **5:**166–169.
15. **Salonen, J. H., E. Eerola, and O. Meurman.** 1998. Clinical significance and outcome of anaerobic bacteremia. Clin. Infect. Dis. **26:**1413–1417.
16. **Snydman, D. R., N. V. Jacobus, L. A. McDermott, R. Ruthazer, Y. Golan, E. J. Goldstein, S. M. Finegold, L. J. Harrell, D. W. Hecht, S. G. Jenkins, C. Pierson, R. Venezia, V. Yu, J. Rihs, and S. L. Gorbach.** 2007. National survey on the susceptibility of *Bacteroides fragilis* group: report and analysis of trends in the United States from 1997 to 2004. Antimicrob. Agents Chemother. **51:**1649–1655.
17. **Snydman, D. R., N. V. Jacobus, L. A. McDermott, R. Ruthazer, E. J. Goldstein, S. M. Finegold, L. J. Harrell, D. W. Hecht, S. G. Jenkins, C. Pierson, R. Venezia, J. Rihs, and S. L. Gorbach.** 2002. National survey on the susceptibility of *Bacteroides fragilis* group: report and analysis of trends for 1997–2000. Clin. Infect. Dis. **35:**S126–S134.
18. **Teng, L. J., P. R. Hsueh, J. C. Tsai, S. J. Liaw, S. W. Ho, and K. T. Luh.** 2002. High incidence of cefoxitin and clindamycin resistance among anaerobes in Taiwan. Antimicrob. Agents Chemother. **46:**2908–2913.
19. **Wybo, I., D. Pierard, I. Verschraegen, M. Reynders, K. Vandoorslaer, G. Claeys, M. Delmee, Y. Glupczynski, B. Gordts, M. Ieven, P. Melin, M. Struelens, J. Verhaegen, and S. Lauwers.** 2007. Third Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria. J. Antimicrob. Chemother. **59:**132–139.