ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1992, p. 2051–2053
0066-4804/92/092051-03$02.00/0

Vol. 36, No. 9

Susceptibilities of Members of the *Bacteroides fragilis* Group to 11 Antimicrobial Agents

RUTH HORN,* JOCELYNE LAVALLÉE, AND HUGH G. ROBSON

Department of Microbiology, Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1

Received 25 March 1992/Accepted 24 June 1992

The susceptibilities of 200 clinical isolates of the *Bacteroides fragilis* group to 11 antimicrobial agents were determined by the broth microdilution method of the National Committee for Clinical Laboratory Standards. All isolates were susceptible to imipenem and ticarcillin-clavulanic acid. The rates of resistance to cefoxitin and clindamycin were low (4 and 6%, respectively), while those to ceftizoxime and cefotetan were higher (10.5 and 24%, respectively).

Members of the *Bacteroides fragilis* group of organisms are the most important and most frequently isolated anaerobes from intra-abdominal, pelvic, and surgical wound infections. In the past few years, several reports (2, 5–10, 12, 13, 16, 18, 22–24) have emphasized the changing susceptibility patterns of the *Bacteroides* species seen within institutions and the difference in resistance rates displayed by the various species. Nationwide surveys have documented increases in resistance rates along with geographic variabilities in resistance rates (8, 9, 12, 13, 22). The National Committee for Clinical Laboratory Standards (NCCLS) has recommended that susceptibility testing be done periodically to monitor resistance patterns in local institutions (19). For this reason, we tested 200 clinical isolates from patients at our hospital against 11 antimicrobial agents.

Nonduplicated clinical isolates of the *B. fragilis* group were collected and frozen during a 22-month period from January 1990 through October 1991. Determination of species was done by using the Minitek system (BBL Microbiology Systems, Cockeysville, Md.).

The following standard antimicrobial powders were kindly provided by the indicated manufacturers: ampicillin, Ayerst Laboratories, St. Laurent, Quebec, Canada; ampicillin-sulbactam, Pfizer Canada Inc., Kirkland, Quebec, Canada; ticarcillin-clavulanic acid and ceftizoxime, SmithKline Beecham Laboratories, Oakville, Ontario, Canada; piperacillin, Lederle Cyanamid Canada Inc., Baie d'Urfé, Quebec, Canada; cefoxitin and imipenem, Merck Frosst Canada Inc., Pointe-Claire, Quebec, Canada; cefotetan, ICI Pharma, Mississauga, Ontario, Canada; clindamycin, The UpJohn Company of Canada, Don Mills, Ontario, Canada; and metronidazole, Rhône-Poulenc Pharma Inc., Montreal, Quebec, Canada. Of these, ampicillin, piperacillin, cefoxitin, clindamycin, and metronidazole were on the Royal Victoria Hospital formulary during the study period. Ticarcillin had been discontinued 2 years earlier, and the others had never been used.

Antimicrobial susceptibility testing was performed by the broth microdilution method recommended by NCCLS (19). Serial twofold dilutions of each antimicrobial agent were made in Anaerobe broth MIC (Difco) with a total volume of 100 μl per well. Colonies were suspended in Anaerobe broth and dispensed to yield a final inoculum of 10^5 CFU per well. All plates were incubated at 35°C for 48 h. The MIC was defined as the lowest concentration of each agent which inhibited the visible growth of the test isolate. If trailing endpoints were observed, the concentration at which the most significant reduction of growth was observed was chosen as the endpoint. With each susceptibility run, controls were included by using *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741. β-Lactamase activity was tested by the nitrocefin method by using nitrocefin disks (Cefinase disks; BBL).

Determination of the species of the 200 strains yielded 100 isolates of *B. fragilis*, 41 *B. distasonis*, 23 *B. vulgatus*, 19 *B. ovatus*, 13 *B. thetaiotaomicron*, 2 *B. uniformis*, and 2 *B. cacae*. The largest number of isolates originated from surgical wounds (35%) and peritoneal fluids (16%). Blood culture isolates accounted for 6.5% of the total number of isolates.

Eighty percent of the isolates tested positive for β-lactamase. This test alone, though, was a poor predictor of resistance, since among these β-lactamase-positive organisms, resistance ranged from only 5% (to cefoxitin) to 21% (to cefotetan). Conversely, the presence of a resistance MIC correlated highly with the presence of β-lactamase.

The susceptibilities of the 200 isolates are given in Table 1. The most active agents were imipenem and ticarcillin-clavulanic acid. No resistance to these antimicrobial agents was detected. Among the β-lactams, ampicillin-sulbactam were the next most active agents, with only 2% of the organisms

TABLE 1. Antimicrobial activities against the *B. fragilis* group

Antimicrobial agent	MIC (μg/ml)[a]			% Resistant[b]	Breakpoint concn (μg/ml)[c]
	Range	50%	90%		
Ampicillin	0.5–>64	16	64	89	4
Ampicillin-sulbactam	0.5–32	2	8	2	16/8
Piperacillin	1–>64	4	32	5.5	64
Ticarcillin	1–>64	32	64	12.5	64
Ticarcillin-clavulanic acid	<0.03–32	0.25	4	0	64/2
Imipenem	<0.03–2	0.12	0.5	0	8
Cefoxitin	1–64	16	32	4	32
Ceftizoxime	0.25–>64	4	64	10.5	32
Cefotetan	1–>64	8	64	24	32
Clindamycin	<0.03–>64	0.5	4	6	4
Metronidazole	0.25–32	1	2	0.5	16

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
[b] Resistance was determined at the breakpoint concentration.
[c] Based on the criteria of NCCLS (19).

* Corresponding author.

TABLE 2. Resistance rates of *B. fragilis* group species

Species (no. of isolates)	% Resistant to the following agents[a]:								
	AMP	AMP-SULB	TIC	PIP	FOX	CZX	CTT	CLN	MTZ
B. fragilis (100)	94	0	10	3	1	6	5	4	0
B. distasonis (41)	80.4	7.3	14.6	7.3	9.7	13.7	58.5	9.7	2.4
B. vulgatus (23)	74	0	17.4	4.3	0	4.3	4.3	8.7	0
B. ovatus (19)	89.5	0	10.5	5.3	5.3	21	58	5.3	0
B. thetaiotaomicron (13)	100	7.7	15.4	15.4	15.4	15.4	38.5	7.7	0

[a] Resistance was determined at the breakpoint concentration. AMP, ampicillin; AMP-SULB, ampicillin-sulbactam; TIC, ticarcillin; PIP, piperacillin; FOX, cefoxitin; CZX, ceftizoxime; CTT, cefotetan; CLN, clindamycin; MTZ, metronidazole. No resistance to imipenem or ticarcillin-clavulanic acid was found.

being resistant to the combination. The addition of sulbactam greatly reduced the rate of resistance to ampicillin alone, which was 94%. Resistance to piperacillin (5.5%) was lower than that to ticarcillin (12.5%). Among the cephalosporin and cephamycin agents, resistance was as follows: 4% for cefoxitin, 10.5% for ceftizoxime, and 24% for cefotetan. Six percent of the isolates were resistant to clindamycin, and only 0.5% were resistant to metronidazole.

There was variability in the resistance rates to some drugs among the various species of the *B. fragilis* group (Table 2). This was seen in particular with the cephalosporin and cephamycin agents. *B. fragilis* and *B. vulgatus* had low rates of resistance; and *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron* had very high rates of resistance to those agents. Clindamycin resistance rates were highest in *B. distasonis* (9.7%) and *B. vulgatus* (8.7%).

The results of our study indicate that, apart from ampicillin and cefotetan, the other agents tested were active against the *B. fragilis* group of organisms, despite a large preponderance of β-lactamase producers. These results are in agreement with those of other published studies (1, 2, 5, 9, 12, 22, 24). Results of this study also confirm the reports (2, 6–9, 12, 18) of others regarding the variation in susceptibility patterns among the species of the *B. fragilis* group.

Data from our study and others (7–9, 12, 18) indicate that the cephalosporins are not equally active against the *B. fragilis* group of organisms. There is considerable variation in the reported rates of resistance to cefoxitin. It is important to remember, however, that methodology, choice of breakpoints, and selection of species are important determinants of results and may vary from one study to another (3, 4, 14). In a yearly national survey done at New England Medical Center from 1981 to 1986, resistance to cefoxitin varied considerably from year to year when compared at a breakpoint of 16 μg/ml, but it was relatively stable at 2 or 3% when a breakpoint of 32 μg/ml (now recommended by the NCCLS) was used (12, 13, 22). However, a nationwide survey in Canada of cefoxitin resistance among *B. fragilis* organisms isolated from clinically significant sites showed an increase in resistance from 2% in 1986 to 26% in 1992 (8, 9). The investigators stated that cefoxitin is no longer reliably active in vitro. Interestingly, our isolates still maintain a low rate of resistance (4%) to cefoxitin. It is important, however, that with cefoxitin, 20% of our isolates fell on the breakpoint and 43% fell within 1 dilution of the breakpoint. Our isolates of *B. thetaiotaomicron* did show a higher rate of resistance (15%), but this was still much lower than that observed in the Canadian survey. As for ceftizoxime and cefotetan, the rates of resistance of 10 and 24%, respectively, found in this study were lower than those of 15 and 53%, respectively, reported in the Canadian survey. The rates of resistance in this study were much closer to those reported by Aldridge and Henderberg (1). Ceftizoxime and cefotetan, in particular, are still not reliably active in vitro.

For years, clindamycin has been considered highly effective against the *B. fragilis* group of organisms, and indeed, it has been seen as a standard with which newer agents can be compared. Resistance to clindamycin began to be reported in the literature by 1980, and since then there has been a slowly progressive increase in resistance worldwide. The recent Canadian survey (9) notes an increase in resistance to clindamycin from 0.7% in 1986 to 9% in 1992. The rate of resistance to clindamycin found in this study (6%) is in agreement with that found in the Canadian survey (9) and with the rates of resistance reported in the most recent U.S. national survey (12). Higher rates of resistance to clindamycin (up to 33%) have been reported by other investigators in the United States (23). Garcia-Rodriguez and Gardia-Sanchez (15) reported a clindamycin resistance rate of 5 to 6% in Spain, while Betriu et al. (7) reported one of 21% and Palaez et al. (20) reported one of 45%. This again emphasizes the institutional variability of susceptibility patterns within cities and countries. Lee et al. (18) also reported a high rate of resistance (24%) to clindamycin in the Republic of Korea.

Fortunately, although resistance to imipenem, metronidazole, and β-lactam–β-lactamase inhibitor combinations has been found, the rates of resistance have been low throughout the world (10, 17, 20, 21).

From the results of this study, it is evident that there are institutional variabilities in the susceptibility patterns of *B. fragilis* and that hospital laboratories must periodically monitor these patterns so that the appropriate antimicrobial agents are available and physicians can make suitable choices when empirically treating anaerobic infections. Moreover, determination of the species and susceptibility testing may be indicated in patients with bacteremias and those with infections in which *B. fragilis* is the sole or predominant organism.

Finally, more studies like that of Brook (11) comparing the in vitro susceptibilities and in vivo efficacies of antimicrobial agents are urgently needed.

This work was supported by grants from Merck Frosst Canada Inc., SmithKline Beecham Canada, and The UpJohn Company of Canada.

REFERENCES

1. **Aldridge, K. E., and A. Henderberg.** 1991. In vitro susceptibility of *Bacteroides fragilis* group strains from abscesses, body fluids and wound/tissue sources, abstr. 702, p. 132. 17th International Congress of Chemotherapy 1991. Futuramed-Verl.-Ges., Munich.
2. **Aldridge, K. E., A. Henderberg, D. D. Schiro, and C. V. Sanders.** 1988. Susceptibility of *Bacteroides fragilis* group isolates to broad-spectrum β-lactams, clindamycin, and metronida-

zole: rates of resistance, cross-resistance, and importance of β-lactamase production. Adv. Ther. **5:**273–282.
3. **Aldridge, K. E., and C. V. Sanders.** 1987. Antibiotic- and method-dependent variation in susceptibility testing results of *Bacteroides fragilis* group isolates. J. Clin. Microbiol. **25:**2317–2321.
4. **Aldridge, K. E., H. M. Wexler, C. V. Sanders, and S. M. Finegold.** 1990. Comparison of in vitro antibiograms of *Bacteroides fragilis* group isolates: differences in resistance rates in two institutions because of differences in susceptibility testing methodology. Antimicrob. Agents Chemother. **34:**179–181.
5. **Appelbaum, P. C., S. K. Spangler, and M. R. Jacobs.** 1991. Susceptibilities of 394 *Bacteroides fragilis*, non-*B. fragilis* group *Bacteroides* species, and *Fusobacterium* species to newer antimicrobial agents. Antimicrob. Agents Chemother. **35:**1214–1218.
6. **Appleman, M. D., P. N. R. Heseltine, and C. E. Cherubin.** 1991. Epidemiology, antimicrobial susceptibility, pathogenicity, and significance of *Bacteroides fragilis* group organisms isolated at Los Angeles County-University of Southern California Medical Center. Rev. Infect. Dis. **13:**12–18.
7. **Betriu, C., E. Campos, C. Cabronero, C. Rodriguez-avial, and J. J. Picazo.** 1990. Susceptibility of species of the *Bacteroides fragilis* group to 10 antimicrobial agents. Antimicrob. Agents Chemother. **34:**671–673.
8. **Bourgault, A. M., G. K. Harding, J. A. Smith, G. B. Horsman, T. J. Marrie, and F. Lamothe.** 1986. Survey of anaerobic susceptibility patterns in Canada. Antimicrob. Agents Chemother. **30:**798–801.
9. **Bourgault, A. M., F. Lamothe, D. Hoban, M. T. Daltone, P. C. Kibsey, G. Harding, J. Smith, D. Low, and H. Gilbert.** 1992. Survey of *Bacteroides fragilis* group susceptibility patterns in Canada. Antimicrob. Agents Chemother. **36:**343–347.
10. **Breuil, J., C. Burnat, O. Patey, and A. Dublanchet.** 1989. A survey of *Bacteroides fragilis* susceptibility patterns in France. J. Antimicrob. Chemother. **24:**69–75.
11. **Brook, I.** 1989. In vitro susceptibility and in vivo efficacy of antimicrobials in the treatment of *Bacteroides fragilis-Escherichia coli* infections in mice. J. Infect. Dis. **160:**651–656.
12. **Cuchural, G. J., Jr., F. P. Tally, N. V. Jacobus, K. Aldridge, T. Cleary, S. M. Finegold, G. Hill, P. Iannini, J. P. O'Keefe, C. Pierson, D. Crook, T. Russo, and D. Hecht.** 1988. Susceptibility of the *Bacteroides fragilis* group in the United States: analysis by site of isolation. Antimicrob. Agents Chemother. **32:**717–722.
13. **Cuchural, G. J., Jr., F. P. Tally, N. V. Jacobus, S. L. Gorbach, K. Aldridge, T. Cleary, S. M. Finegold, G. Hill, P. Iannini, J. P. O'Keefe, and C. Pierson.** 1984. Antimicrobial susceptibilities of 1,292 isolates of the *Bacteroides fragilis* group in the United States: comparison of 1981 with 1982. Antimicrob. Agents Chemother. **26:**145–148.
14. **Finegold, S. M.** 1988. Susceptibility testing of anaerobic bacteria. J. Clin. Microbiol. **26:**1253–1256.
15. **Garcia-Rodriguez, J. E., and J. E. Gardia-Sanchez.** 1990. Evolution of antimicrobial susceptibility in isolates of the *Bacteroides fragilis* group in Spain. Rev. Infect. Dis. **12**(Suppl. 2)**:**S142–S151.
16. **Goldstein, E. J. C., and D. M. Citron.** 1988. Annual incidence, epidemiology and comparative in vitro susceptibilities to cefoxitin, cefotetan, cefmetazole, and ceftizoxime of recent community-acquired isolates of the *Bacteroides fragilis* group. J. Clin. Microbiol. **26:**2361–2366.
17. **Hurlbut, S., G. J. Cuchural, and F. P. Tally.** 1990. Imipenem resistance in *Bacteroides distasonis* mediated by a novel β-lactamase. Antimicrob. Agents Chemother. **34:**117–120.
18. **Lee, K., I. H. Jang, Y. J. Kim, and Y. Chong.** 1992. In vitro susceptibilities of the *Bacteroides fragilis* group to 14 antimicrobial agents in Korea. Antimicrob. Agents Chemother. **36:**195–197.
19. **National Committee for Clinical Laboratory Standards.** Methods for antimicrobial susceptibility testing of anaerobic bacteria, 2nd ed. Approved standard. NCCLS publication no. M11-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
20. **Palaez, M. T., E. Cercendo, M. Rodriguez-Creixems, and E. Bouza.** 1991. Letter. Rev. Infect. Dis. **13:**183.
21. **Scher, K. S.** 1988. Emergence of antibiotic resistant strains of *Bacteroides fragilis*. Surg. Gynecol. Obstet. **167:**175–179.
22. **Tally, F. P., G. J. Cuchural, Jr., N. V. Jacobus, S. L. Gorbach, K. Aldridge, T. Cleary, S. M. Finegold, G. Hill, P. Iannini, J. P. O'Keefe, and C. Pierson.** 1985. Nationwide study of the susceptibility of the *Bacteroides fragilis* group in the United States. Antimicrob. Agents Chemother. **28:**675–677.
23. **Wexler, H. M., and S. M. Finegold.** 1987. Antimicrobial resistance to Bacteroides. J. Antimicrob. Chemother. **19:**143–146.
24. **Wexler, H. M., and S. M. Finegold.** 1988. In vitro activity of cefotetan compared with that of other antimicrobial agents against anaerobic bacteria. Antimicrob. Agents Chemother. **32:**601–604.