ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1990, p. 179–181
0066-4804/90/010179-03$02.00/0

Vol. 34, No. 1

Comparison of In Vitro Antibiograms of *Bacteroides fragilis* Group Isolates: Differences in Resistance Rates in Two Institutions because of Differences in Susceptibility Testing Methodology

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Received 7 March 1989/Accepted 25 October 1989

With 120 clinical isolates of the *Bacteroides fragilis* group, a comparison of rates of resistance to selected antimicrobial agents by using two susceptibility tests was performed in two medical institutions. The broth microdilution method produced MICs significantly lower than those determined by the agar dilution method. With ceftizoxime and cefoxitin, 88 and 18%, respectively, of the MICs were ≥2 twofold dilutions apart. These differences in MIC results produced major interpretive discrepancies for ceftizoxime and cefoxitin, whereas no significant differences in resistance rates were noted for clindamycin and metronidazole.

An increased incidence of resistance of *Bacteroides fragilis* group isolates to beta-lactam and other antimicrobial agents has been noted in the United States as well as in other countries (3, 4, 20). Among *B. fragilis* group strains, resistance to beta-lactam antibiotics is mediated primarily by β-lactamase production; however, resistance due to the production of altered penicillin-binding proteins has been described (21).

Cefoxitin resistance has increased within the last 10 years in the United States and varies geographically (20). Other cephalosporins, such as cefotetan, cefoperazone, cefamandole, cefuroxime, and cefotaxime, have only moderate to poor activities against *B. fragilis* group isolates (2, 20). The activity of ceftizoxime, an aminothiazolyl methoxyimino cephalosporin, against anaerobes, particularly against the *B. fragilis* group, has been controversial, and different resistance rates have appeared in the literature (2, 5, 7, 8, 14, 18).

The present study was performed (i) to compare the activities of ceftizoxime, cefoxitin, clindamycin, and metronidazole against *B. fragilis* group isolates in two institutions, using different susceptibility testing methods; (ii) to exchange an equal number of *B. fragilis* group isolates for retesting; and (iii) to determine the influence of testing methodology on the activity of the antimicrobial agents being studied.

Sixty clinical isolates of the *B. fragilis* group were tested in the routine manner at each participating institution. The 60 isolates were then exchanged between institutions and retested according to the routine testing procedure of each laboratory. The following numbers of strains were used: *B. fragilis*, 60; *Bacteroides thetaiotaomicron*, 24; *Bacteroides distasonis*, 19; and *Bacteroides ovatus*, 17. For the studies with clindamycin and metronidazole, only 60 isolates were tested and were represented in the same proportions of the various species as described above. Each isolate was identified by using gas-liquid chromatography and biochemical profiles (6, 11, 19).

Two methods were used for testing the antimicrobial agent susceptibility of each isolate. One method, routinely performed at Louisiana State University Medical Center, was a broth microdilution method (BMD), as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (15). Serial twofold dilutions of each antimicrobial agent (0.125 to 256 μg/ml) were prepared in Anaerobe broth (Anaerobe broth MIC; Difco Laboratories, Detroit, Mich.) supplemented with vitamin K_1 (0.5 μg/ml) and hemin (5 μg/ml) and dispensed into microdilution wells, with a final volume of 100 μl per well. The inoculum was prepared by inoculating 3 to 5 colonies of the test organism into a tube of prereduced Anaerobe broth supplemented with vitamin K_1 (0.5 μg/ml) and hemin (5 μg/ml) and incubating for 3 to 6 h anaerobically at 35°C. The organism suspension was adjusted to the density of a 0.5 McFarland standard and then further diluted to give a final inoculum size of approximately 10^5 CFU per well upon delivery of 1 to 2 μl with a semiautomated inoculator (Dynatech Industries, Inc., McLean, Va.). Colonies were counted on the final inoculum of one clinical isolate from each run to ensure an appropriate inoculum size. All plates were incubated in an anaerobic chamber for 48 h at 35°C before being read. The MIC was defined as the lowest concentration of antimicrobial agent inhibiting the visible growth of the test organism. Trailing endpoints have been observed with some antimicrobial agents (17). If this occurred, the concentration at which the most significant reduction of growth was observed was the MIC endpoint. The second method, routinely performed at the Veterans Administration Wadsworth Medical Center, was an agar dilution method (AD) using a different test medium than that recommended by the NCCLS (16); however, the other test parameters were based on NCCLS recommendations. Serial twofold dilutions of each antimicrobial agent (0.063 to 256 μg/ml) were prepared in melted brucella agar (GIBCO Laboratories, Grand Island, N.Y.) supplemented with vitamin K_1 (10 μg/ml) and laked sheep blood (5%). The inoculum was prepared by transferring 3 to 5 colonies of the test organism to prereduced thioglycolate broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with vitamin K_1 (0.1 μg/ml) and incubating aerobically at 35°C for 3.5 to 4 h. The organism suspension was then adjusted in brucella broth to the turbidity of a 0.5 McFarland standard. Each isolate was loaded in the inoculum block of a Steers replicator, and with the multipoint inoculator 1 to 3 μl of each suspension was delivered to the surface of each plate, giving a final inoculum size of approximately 10^5 CFU per spot. The plates were

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TABLE 1. Comparison of activities of various antimicrobial agents against strains of the *B. fragilis* group as determined by BMD and AD

Antibiotic (no. of isolates tested) and test method	MIC (μg/ml)[a]			% Susceptible at the following concn (μg/ml):					
	Range	50%	90%	2	4	8	16	32	64
Ceftizoxime (120)									
BMD	0.125–25	4	32			83	88	94	98
AD	1–512	64	128			9	18	42	73
Cefoxitin (120)									
BMD	1–64	16	32			40	75	94	100
AD	8–128	32	64			6	47	71	97
Clindamycin (60)									
BMD	0.063–128	0.25	2	92	95	97			
AD	0.125–>256	1	8	80	85	90			
Metronidazole (60)									
BMD	0.25–2	1	2			100	100	100	
AD	1–8	4	4			100	100	100	

[a] 50% and 90%, MIC for 50 and 90% of isolates tested, respectively.

incubated for 48 h at 37°C in anaerobic jars (BBL) before being read. The MIC was defined as the lowest concentration of each antimicrobial agent which yielded no growth, 1 to 2 colonies, or a faint haze.

Each of the following antimicrobial agents was kindly provided by the manufacturer: ceftizoxime (Smith Kline & French Laboratories, Philadelphia, Pa.), cefoxitin (Merck Sharp & Dohme, West Point, Pa.), clindamycin (The Upjohn Co., Kalamazoo, Mich.), and metronidazole (G. D. Searle, Chicago, Ill.). Resistance to ceftizoxime, cefoxitin, and metronidazole was judged at concentrations of 8, 16, 32, and 64 μg/ml, while resistance to clindamycin was judged at 2, 4, and 8 μg/ml.

When BMD was used, ceftizoxime and cefoxitin had significantly more activity than when tested by AD (Table 1). When BMD was used, the ceftizoxime MICs for 50 and 90% of the strains tested were 16- and 4-fold lower, respectively, than those obtained with AD, whereas these values were only 2-fold lower when cefoxitin was tested. At a breakpoint of ≥64 μg/ml, the rates of resistance to ceftizoxime were 6% by BMD and 58% by AD, and the rates of resistance to cefoxitin were 6% by BMD and 29% by AD. *B. fragilis* was more susceptible to ceftizoxime and cefoxitin than were the strains of the non-*B. fragilis* species by both BMD and AD (data not shown in Table 1). Ceftizoxime inhibited 87, 88, 92, and 97% of the *B. fragilis* strains at 8, 16, 32, and 64 μg/ml, respectively, when tested by BMD, whereas 5, 13, 32, and 73% of these strains were inhibited by the same concentrations when tested by AD. For non-*B. fragilis* strains, ceftizoxime inhibited 78, 88, 97, and 98% of the strains at 8, 16, 32, and 64 μg/ml, respectively, as determined by BMD and 15, 23, 52, and 73% of the strains at the same concentrations by AD. Cefoxitin inhibited 63, 95, 98, and 100%, respectively, of the *B. fragilis* strains at 8, 16, 32, and 64 μg/ml by BMD and 77, 88, 97, and 98%, respectively, by AD. Similarly, for non-*B. fragilis* strains cefoxitin inhibited 17, 55, 90, and 100% of the strains at 8, 16, 32, and 64 μg/ml, respectively, by BMD and inhibited 3, 20, 45, and 95% of the strains at the same concentrations by AD. With cefoxitin, the MICs for the majority of the strains clustered at 8 and 16 μg/ml as determined by BMD and clustered at 16, 32, and 64 μg/ml as determined by AD. For clindamycin, the MICs generated by BMD were generally two- to fourfold lower than AD results. In addition, resistance rates were not as dramatically affected by the two methods. For metronidazole, the overall differences were least affected by the method used.

When the differences in the MIC endpoints generated by the two methods were compared, the greatest number of endpoint discrepancies between the two methods was seen when testing ceftizoxime. In 88% of the comparisons, the ceftizoxime endpoints were fourfold or more apart; with cefoxitin, the endpoints determined by the two methods were equal to or within 1 twofold dilution of each other 82% of the time. Clindamycin showed a wider distribution of endpoint differences and tended to be species dependent, with the greater number of MIC endpoint differences noted for *B. fragilis* and *B. distasonis*. With metronidazole, the majority of the MIC endpoints (72%) were equal to or within 1 twofold dilution of each other when the two methods were compared.

Table 2 compares the number of major discrepancies noted when the MICs were used to determine the various susceptibility categories. Ceftizoxime produced the greatest number of major discrepancies, with 53% of the isolates being susceptible by BMD and resistant by AD at a cutoff point of ≥64 μg/ml. For cefoxitin, the two methods produced a major discrepancy rate of 22% at ≥64 μg/ml. Major discrepancies were low (12%) for clindamycin, and none were observed with metronidazole.

This study has shown that the in vitro resistance rates of *B. fragilis* group isolates can be significantly influenced by

TABLE 2. Comparison of major interpretive discrepancies due to MIC differences by BMD and AD

Antibiotic	Resistance concn (μg/ml)[a]	No. of strains (%) for which major discrepancies were seen[b]
Ceftizoxime	≥64	63/120 (53)
Cefoxitin	≥64	27/120 (22)
Clindamycin	≥8	8/60 (12)
Metronidazole	≥16	0/60 (0)

[a] Based on NCCLS recommendations (17).

[b] A major interpretive discrepancy occurred when the MIC was judged as indicating resistance by one method whereas the other method gave a susceptibility MIC.

the testing methodology. Of the compounds tested, ceftizoxime was the most affected by the different methods. Thus, what appeared to be significant differences in resistance rates for different organism populations at two separate medical institutions is the result of differences in methodology. The resistance rates of cefoxitin were also methodology dependent, showing much lower resistance rates by BMD. The cefoxitin MIC endpoints as determined by both methods tended to cluster around 16, 32, or 64 μg/ml, and a methodology-dependent change of 1 or 2 twofold dilutions in MIC endpoints can significantly influence the resistance rates, as noted in the present study. Similar results for ceftizoxime and cefoxitin have also been reported by Jones et al. (12). Although some endpoint differences were seen when clindamycin and metronidazole were tested, they did not have a significant influence on the overall rates of resistance to these compounds. In addition, Aldridge and Sanders (1) compared the NCCLS reference AD method with a comparable BMD test for all four antimicrobial agents and found results very similar to those reported here.

Because of the wide disparity of results between the two methods, the question arises of which method is more predictive of the clinical outcome in patients. Unfortunately, this question is unanswered at this point, and prospective studies are needed. Clinical studies with cefoxitin and ceftizoxime in certain types of infections involving anaerobes have been reported (9, 10, 13; Sr. M. A. Lou, E. Valdepenas, J. Mackabee, M. Wikler, and J. McDevitt, Proc. 14th Int. Congr. Chemother., p. 146, 1985), but a comparison of different susceptibility testing methods was not undertaken. Clearly, the clinical outcome of an infection is influenced not only by in vitro susceptibility tests but also by other factors such as surgery, achieving adequate drug levels at the infected site, the severity of the infection, the immunocompetence of the patient, and additional underlying diseases of the patient.

In conclusion, it is important that new attempts at standardizing a method for susceptibility testing of anaerobic bacteria be undertaken with the goal of minimizing the differences produced by different testing methods and thus eliminating what appear to be significant differences in the resistance rates between institutions. Until then, a comparison of resistance rates for certain antimicrobial agents among institutions must also include a consideration of the testing methodology used.

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