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Bacteroides fragilis Resistance to Clindamycin In Vitro

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Clindamycin resistance in *Bacteroides fragilis* was examined in 507 strains isolated from 1973 to 1981. Three groups were recognized: highly susceptible (minimum inhibitory concentration [MIC] ≤ 0.125 μg/ml), intermediately susceptible (MIC = 0.25 to 4 μg/ml), and highly resistant (MIC ≥ 8 μg/ml). The incidence of high-level resistance (1.8%) had not changed during this period. Only 8 of 17 isolates reputed to be highly clindamycin resistant that were referred to our laboratory proved to be highly resistant (MICs ≥ 32 μg/ml), whereas the other 9 were intermediately susceptible. Analysis of 2- and 10-μg clindamycin disks for determining the susceptibility of *B. fragilis* revealed a high false-resistance rate with the 2-μg disk, most errors occurring with the intermediate group. There was no false resistance with the 10-μg disk. When disk diffusion susceptibility of *B. fragilis* is employed, we recommend the 10-μg disk to predict accurately the susceptibility of *B. fragilis* to clindamycin.

Clindamycin is an important antimicrobial agent for the therapy of anaerobic infections because of its consistent activity against *Bacteroides fragilis* and other pathogenic anaerobic bacteria (3). Since the early 1970s, there has been relatively little resistance to clindamycin among these organisms (6, 10). Recent studies from four hospitals in Detroit, Mich., however, have reported 15 to 20% incidence of clindamycin resistance in *Bacteroides* species (1). Furthermore, three laboratories have documented transferable clindamycin resistance in these organisms (7, 16, 18). These observations suggest that antimicrobial resistance in *Bacteroides* species should be monitored closely in clinical microbiology laboratories.

A problem in such monitoring has been the lack of a standardized susceptibility method for anaerobic bacteria and the great variability among the methods described, i.e., disk diffusion, disk broth dilution, agar dilution, and broth dilution. Many laboratories utilize the Bauer-Kirby method of disk diffusion for testing aerobic and facultative bacteria, and this test has been applied to anaerobic bacteria as well (2, 12). Unfortunately, the disk diffusion method, when applied to anaerobic microorganisms, has many limitations (14). In addition to the inherent technical problems, the content of clindamycin in the disk is an issue. The 2-g clindamycin disk has U.S. Food and Drug Administration approval for routine susceptibility testing, but we have found that it gives a falsely high rate of clindamycin resistance in *B. fragilis*.

One purpose of this study was to determine whether there has been any trend toward increased clindamycin resistance in *B. fragilis* in the past 8 years in our hospital. We also wished to test the reliability of the disk diffusion method for predicting clindamycin susceptibility of the *B. fragilis* group of organisms and to analyze strains referred to our laboratory for reputed clindamycin resistance.

MATERIALS AND METHODS

Antibiotic. Clindamycin hydrochloride standard powder and susceptibility disks containing 2 and 10 μg of clindamycin were obtained from The Upjohn Co., Kalamazoo, Mich. Antimicrobial solutions were prepared immediately before each series of tests in sterile distilled water.

Organisms. A collection of 507 strains belonging to the *B. fragilis* group, which includes *B. fragilis*, *B. thetaiotaomicron*, *B. ovatus*, *B. distasonis*, and *B. vulgatus*, were studied to determine the incidence of clindamycin resistance. The organisms were isolated from clinical specimens in our laboratory, first at the Sepulveda Veterans Administration Hospital, Sepulveda, Calif., from 1973 to 1975, and subsequently at the Tufts Anaerobic Bacteriology Research Laboratory of the New England Medical Center, Boston, Mass., from 1975 to 1981. Each isolate was obtained from a different patient, unless there was a change in the antimicrobial susceptibility pattern of the organism in the same patient. The strains were identified according to previously established criteria (4).

To test the reliability of the disk diffusion method for predicting susceptibility, 126 strains of *B. fragilis* were selected to give a range of susceptibility that included highly susceptible strains (minimum inhibitory con-

TABLE 1. Incidence of clindamycin resistance in *Bacteroides* species[a]

Year	No. of strains	No. of resistant strains	% Resistance[b]
1973	38	1	2.6
1974	60	1	1.7
1975	21	0	0.0
1976	16	0	0.0
1977	114	2	1.7
1978	66	1	1.5
1979	30	1	3.3
1980	66	3	4.5
1981	96	0	0.0

[a] Includes *B. fragilis*, *B. thetaiotaomicron*, *B. distasonis*, *B. ovatus*, and *B. vulgatus*.

[b] Organisms resistant to ≥8 μg of clindamycin per ml.

centration [MIC] ≤ 0.125 μg/ml), intermediately susceptible strains (MIC = 0.25 to 4 μg/ml), and resistant strains (MIC ≥ 8 μg/ml).

Seventeen *Bacteroides* isolates were referred to us from other hospitals for presumed resistance to clindamycin. These strains were reidentified, and their antimicrobial susceptibility was tested.

Susceptibility tests. MICs were determined by a modified agar dilution method by using Steer's replicator to inoculate approximately 10^4 colony-forming units per spot onto brain heart infusion agar supplemented with 5% laked sheep blood and vitamin K (10 μg/ml) (2, 11). The inoculum was incubated for 4 to 6 h in supplemented brain heart infusion broth. Anaerobic chamber techniques with an atmosphere of 85% N_2–10% H_2–5% CO_2 were used throughout (15). The results of the agar dilution test were read after 18 h of incubation. The MIC was recorded as the lowest concentration of drug at which there was no growth.

For the disk diffusion test, 4- to 6-h cultures were diluted to a density of one-half of the turbidity of the no. 1 McFarland standard, as recommended by Sutter et al. (13). The inoculum was applied by swabbing in three directions with a cotton swab to freshly prepared brucella agar plates (brucella agar base enriched with 5% defibrinated sheep blood, 0.0005% hemin, and 0.0001% vitamin K). After drying for 3 to 5 min, 2- and 10-μg disks of clindamycin were applied on each plate with sterile forceps. Each organism was tested in triplicate. All plates were incubated at 37°C in an anaerobic chamber. After 18 h of incubation, the zones of inhibition around the disks were read with Vernier calipers.

The MIC and zone of inhibition values were analyzed by the error rate-bounded method as described by Metzler and DeHaan (7). This method has been recommended to analyze data that show great variations about the regression line, as is the case when anaerobic bacteria are tested against clindamycin. A MIC breakpoint of 6 μg/ml was chosen to divide the strains into susceptible and resistant groups. (This concentration is readily achievable in serum with ordinary doses of clindamycin.) A maximal tolerable rate of false susceptible of 1% and a maximal tolerable rate of false resistance of 5% were assigned.

RESULTS

Incidence of clindamycin resistance in *B. fragilis* group. The susceptibility of *Bacteroides* species to clindamycin was analyzed in 507 strains isolated from 1973 to 1981 (Table 1). The overall incidence of resistance (MIC breakpoint ≥ 6 g/ml) for the 8-year period was 1.8%, with a range of 0 to 4.5% per year. Over this period, six highly resistant strains (MIC > 32 μg/ml) were isolated.

Analysis of referred strains. Seventeen isolates of the *B. fragilis* group were referred to our laboratory from 1978 to 1980. These strains had been characterized as resistant by the disk diffusion technique with a 2-μg clindamycin disk. Clindamycin resistance was confirmed in eight strains, for which the MICs were greater than 32 μg/ml. The remaining nine strains had MICs ranging from 0.125 to 2 μg/ml; this range would be considered indicative of susceptibility to clindamycin, based on achievable blood levels.

Disk diffusion as a method to predict susceptibility. The comparison of suceptibility by MIC and by agar diffusion with 2- and 10-μg disks is given on Table 2. Ninety-five percent of highly susceptible strains (MIC ≤ 0.125) were predicted with the 2-μg disk, resulting in false prediction of resistance of 5%. When the 10-μg disk was used, the prediction of susceptibility was 100%. The susceptibility of only 30% of the intermediately susceptible group (MIC = 0.25 to 4 μg/ml) could be predicted with the 2-μg disk, whereas the 10-μg disk gave 100% predictability in this group. The organisms in this intermediate group that appeared susceptible with the 2-μg disk usually had a MIC of about 0.25 μg/ml, whereas those with MICs of 0.5 to 4 μg/ml usually gave zone sizes of less than 15 mm, thereby seeming to be resistant. When resistant organisms (MIC ≥ 8 μg/ml) were tested, there was no false susceptibility with the 2- or 10-μg disks.

Figure 1 shows the analysis of these data by the error rate-bounded method, with a 15-mm-diameter zone of inhibition as the breakpoint for susceptibility with a 10-μg disk. With this break-

TABLE 2. Correlation between MIC by agar dilution and susceptibility by agar diffusion

No. of strains	MIC (μg/ml)	% strains susceptible[a] to clindamycin disks of:	
		2 μg	10 μg
80	≤0.125	95	100
37	0.25–4.0	30	100
9	≥8.0	0	0

[a] Zone diameters of 15 mm or more.

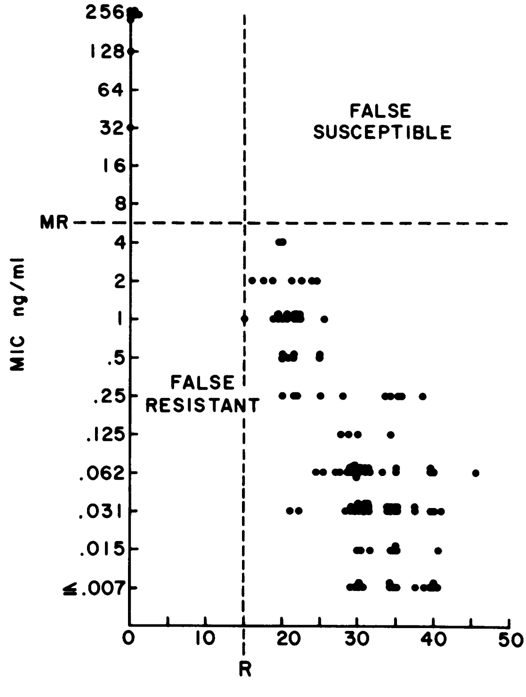

FIG. 1. Classification scheme for zone diameters (10-μg clindamycin disk) obtained by the error rate-bounded method. MR, MIC values chosen as breakpoint; R, critical zone diameters (breakpoint).

point zone diameter, no false-resistant or false-susceptible strains were observed.

DISCUSSION

Resistance of *B. fragilis* to antimicrobial agents appears to have increased in recent years. Tetracycline resistance is found in 50 to 60% of current isolates, even though in the 1950s it was the drug of choice for treating infections involving this organism. More alarming has been the identification of clindamycin-resistant strains, first noted in 1976 (9). In addition to scattered reports of occasional isolates of clindamycin-resistant *Bacteroides* species, there has been a recent report of an 8 to 20% incidence of clindamycin resistance in hospitals in Detroit (1).

Transferable clindamycin resistance has been demonstrated in *B. fragilis*, which can transfer within *B. fragilis* and to other *Bacteroides* species (16, 18). To date, there are at least two known clindamycin resistance transfer factors: one, isolated in our laboratory, is called pBFTM10, and the second, characterized by Welch and Macrina and by Magot et al., is called pBF4 or pIP410, respectively (5, 17, 19). It is known that such genetic systems in aerobic bacteria are responsible for increased resistance of these organisms to multiple antibiotics; therefore, it should be anticipated that antibiotic pressure will produce an increasing resistance to clindamycin in *Bacteroides* species.

Since erythromycin also stimulates clindamycin resistance (9, 17), the use of erythromycin for atypical pneumonias and as a nonabsorbable agent for bowel preparation may prompt an increased incidence of the macrolide-lincosamide-streptogramin resistance in *B. fragilis*. However, the data from our study indicate that there has been no change in the susceptibility of *Bacteroides* species to clindamycin at the New England Medical Center during the past 6 years, from 1975 to 1981, despite increased use of erythromycin, as well as continued use of clindamycin in treating surgical patients. Nevertheless, we would still recommend that selected isolates of *Bacteroides*, such as those from blood cultures, be tested to detect resistance, since alternative therapeutic regimes are available.

Our analysis of these *Bacteroides* strains suggests that they fall into three groups: highly susceptible, intermediately susceptible, and highly resistant. Although the number of strains in the intermediate group is relatively small, these organisms are frequently labeled resistant to clindamycin with the 2-μg disk, when they are actually susceptible to achievable drug levels. The 2-μg disk appears to be inadequate for testing *B. fragilis* susceptibility, and efforts should be made to obtain 10-μg disks for such testing.

Alternatives to the disk method are the broth disk elution method, agar dilution, or the MIC determination by macro- or microbroth dilution. These techniques are suitable for determining the susceptibility of *B. fragilis* to clindamycin. The currently recommended method of the National Committee for Clinical Laboratory Standards is a modification of the agar dilution method in which the different variables of the test are carefully controlled (8). Our current practice is to use either agar dilution or the microbroth dilution technique to determine the susceptibility of clinical isolates. Whatever method is chosen to determine the susceptibility of *Bacteroides* species, the test should be run with appropriate controls and should be correlated with an accepted reference method, such as agar dilution.

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