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Antibiotic Susceptibility Testing of *Bacteroides*

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Seventy *Bacteroides* strains isolated from clinical materials were tested in vitro against nine antibiotics by means of modified disc diffusion and agar dilution tests. Correlating the agar dilution susceptibility with the concentrations achievable in serum for each drug tested, we found that at least 90% of the strains were susceptible to clindamycin, chloramphenicol, carbenicillin, and lincomycin. Only 40% were susceptible to tetracycline. Except for tetracycline and chloramphenicol, the disc diffusion technique exhibited inadequate predictive value in terms of "susceptibility" and "resistance" for the *Bacteroides* strains tested.

Current awareness of the frequency and severity of *Bacteroides* infections has generated interest in antimicrobial agents effective against these anaerobic, non-spore-forming, gram-negative bacilli. The vast majority of *Bacteroides* strains currently isolated from clinical materials are resistant to penicillin as well as to drugs commonly employed for the treatment of gram-negative infections. The search for more appropriate antimicrobial agents has been hampered by the lack of a standardized technique for anaerobic antibiotic susceptibility determinations.

The purposes of the present study were to assess the in vitro effectiveness of various currently available antibiotics against 70 recent *Bacteroides* isolates and to examine the possible application of a standardized single high-potency disc susceptibility testing technique to *Bacteroides* (1).

MATERIALS AND METHODS

Seventy strains of *Bacteroides* were isolated from clinical materials in the general clinical bacteriology laboratory at Vanderbilt University Hospital from January 1970 through May 1971. All were obligate anaerobic gram-negative bacilli of relatively regular shape with rounded ends. Organisms were speciated according to described criteria (4, 8), and all but four were classifiable as members of the "*B. fragilis group*" (*B. fragilis, B. incommunis, B. oralis,* and *B. variabilis*). All organisms were stored at −30 C as 1-day-old colonies on 5% sheep blood-agar squares. Each organism was tested for susceptibility to nine antibiotics by agar plate dilution and high-potency disc diffusion tests, and the results were compared.

Disc diffusion tests. Single high-potency disc diffusion tests were performed according to the method of Bauer et al. (1) with minor modifications. The suspension of organisms used for the inoculum was prepared as follows. After growth for 24 hr on beef heart infusion agar containing 5% defibrinated sheep blood, organisms were suspended in beef heart infusion broth, and turbidity was adjusted to that of a standard prepared with 0.5 ml of 1% $BaCl_2$ and 99.5 ml of 1% H_2SO_4. This suspension was swabbed over the surface of Mueller-Hinton agar plates containing 5% sheep blood and allowed to dry for 15 min, after which currently available high-potency discs were firmly placed on the surface of the inoculated agar. After incubation at 37 C for 48 hr in GasPak anaerobic jars (BBL), the diameters of complete inhibition of bacterial growth about each disc were measured and recorded. If there was no inhibition zone, 6 mm, the disc diameter, was recorded. The antibiotics employed with their disc potencies were as follows: carbenicillin (50 μg), cephalothin (30 μg), chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), lincomycin (2 μg), penicillin (10 units) tetracycline (30 μg), and vancomycin (30 μg).

Agar dilution tests. Beef heart infusion agar was melted and maintained in a liquid state at 47 to 50 C. A solution containing a known quantity of antibiotic was added to beef heart infusion broth, and serial twofold dilutions were made. Each dilution was added to a separate container of melted agar along with 5% defibrinated sheep blood. After gentle mixing, 20 ml of this mixture was poured into 9-cm petri dishes and allowed to harden. Each plate, thus prepared, contained a specific concentration of antibiotic. Plates were refrigerated until used, and all tests were performed within 72 hr of their preparation. Suspensions of the *Bacteroides* isolates were prepared as follows: colonies, grown on sheep blood agar for 24 to 36 hr, were suspended in beef heart infusion broth to the turbidity of the $BaCl_2$ standard described above (approximately 10^7 organisms). A further 10-fold dilution of each was made and transferred to cups arranged complementary to the 19 prongs of a replication apparatus

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similar to the Steers' replicator (7, 10). Each plate in the series was then spot-inoculated with the replicating device. The drop delivered by each prong contained approximately 5×10^4 organisms and covered an area 7 mm in diameter. All plates were incubated at 37 C in GasPak anaerobic jars and were examined for growth at 48 hr. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic within the agar which inhibited visible bacterial growth.

RESULTS

Figure 1 shows the MIC range for each of the nine antimicrobial agents tested against the 70 *Bacteroides* strains. Weight for weight, clindamycin, lincomycin, and chloramphenicol appeared to be the most active agents. More than 90% of the *Bacteroides* strains were inhibited by 3.1 μg of clindamycin/ml, 6.25 μg of lincomycin/ml, and 12.5 μg of chloramphenicol/ml. However, the actual susceptibility or resistance of a microorganism to an antimicrobial agent is best assessed in light of the usual concentrations achievable in serum during clinical use of the drug. Table 1 illustrates these relationships. The first column indicates the highest inhibitory concentration considered compatible with antimicrobial susceptibility based on the usual concentrations achievable in serum after administration of acceptable doses of each drug. With antibiotics which can be administered orally or parenterally, the values are expressed in terms of usual oral doses, except with penicillin, for which a moderate parenteral dose of aqueous penicillin is assumed. In Table 1, the 70 strains of *Bacteroides* are classi-

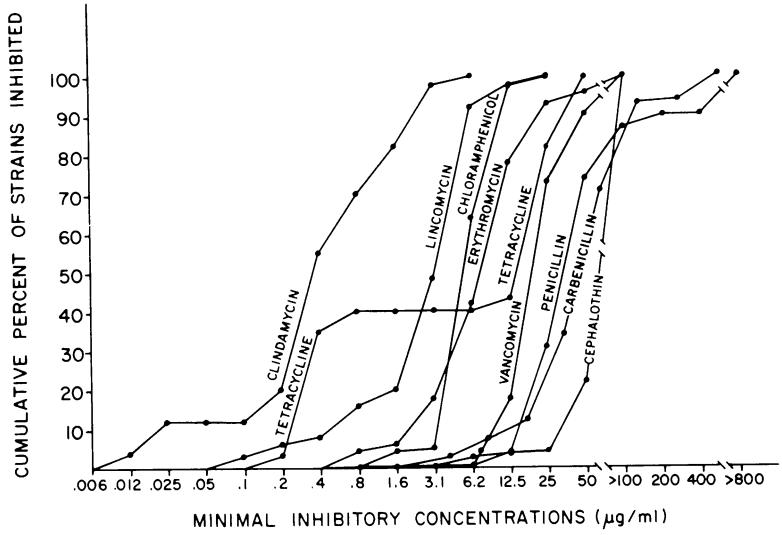

FIG. 1. *Cumulative per cent inhibition of 70 Bacteroides strains by nine different antimicrobial agents. The minimal inhibitory concentration of penicillin is given in units per milliliter.*

TABLE 1. *Antibiotic susceptibility of 70 strains of Bacteroides*

Antibiotic[a]	Susceptible strains			Resistant strains		
	No.	MIC (μg/ml)	Zone size (mm)	No.	MIC (μg/ml)	Zone size (mm)
Clindamycin (3.1 μg/ml)	68	0.006–3.1[b]	8–50	2	6.3	6
Chloramphenicol (12.5 μg/ml)	68	1.6–12.5	20–38	2	25+	14–22
Carbenicillin (125 μg/ml)	65	3.9–125	6–30	5	250–500+	6–10
Lincomycin (6.25 μg)ml)	63	0.125–6.25	6–50	7	12.5–25	6
Erythromycin (6.25 μg/ml)	28	0.78–6.25	12–40	42	12.5–100+	6–37
Tetracycline (6.3 μg/ml)	24	0.095–0.78	25–48	46	12.5–50	6–15
Penicillin (26 units/ml)	22	6.5–26[c]	6–18	48	52–415+[c]	6–32
Vancomycin (12.5 μg/ml)	12	6.25–12.5	18–22	58	25–100+	6–30
Cephalothin (12.5 μg/ml)	3	1.56–12.5	14–32	67	50–100+	6–22

[a] Concentrations given in parentheses represent the highest MIC compatible with susceptibility.
[b] Values given for the MIC and the zone of inhibition represent ranges.
[c] Units.

fied as susceptible or resistant depending on the relationship of their MIC to these values. On this basis, more than 90% of the 70 *Bacteroides* strains could be considered susceptible to clindamycin, chloramphenicol, carbenicillin, and lincomycin. As can be noted, the majority of these strains were resistant to erythromycin, tetracycline, penicillin, vancomycin, and cephalothin.

Disc diffusion results varied considerably for each antibiotic tested and are also shown in Table 1. It can be seen that prediction of the susceptibility of these *Bacteroides* strains to many of the antibiotics on the basis of disc diffusion testing alone was likely to be hazardous because of poor correlation between inhibition zone size and MIC. Notable exceptions were chloramphenicol (only one strain showed poor correlation, being inhibited by 25 μg/ml but showing a 22-mm zone of inhibition) and tetracycline, with which prediction of "susceptibility" or "resistance" from disc diffusion data appeared reliable. There was a bimodal distribution of susceptibility to tetracycline in that 40% of the strains were inhibited by concentrations considerably below "usual" serum levels, whereas 60% required concentrations which would be difficult to obtain with the usual doses of this drug and were hence "resistant."

These relationships are well illustrated in Fig. 2 through 6, in which the MIC is plotted on the ordinate, the disc diffusion inhibition diameter is plotted on the abscissa, and a simple linear regression curve is constructed by the method of least squares ($y = ax + b$). Figure 2 clearly demonstrates the bimodal pattern of activity of tetracycline against the 70 *Bacteroides* isolates, as well as the excellent correlation of zone diameter and MIC values. Similarly, Fig. 3 shows an excellent correlation between the two susceptibility testing techniques for chloramphenicol.

Figures 4, 5, and 6 illustrate a poor correlation

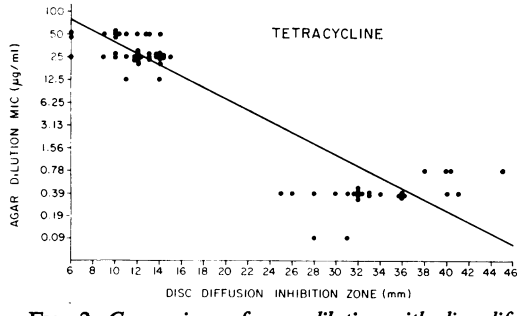

FIG. 2. *Comparison of agar dilution with disc diffusion susceptibility tests for 70 Bacteroides strains against tetracycline.*

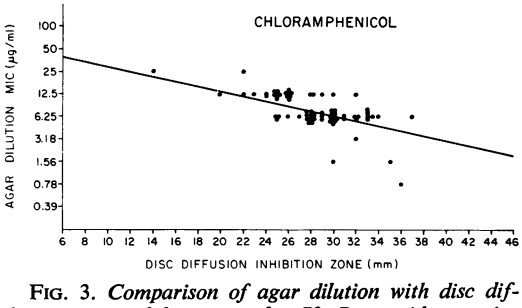

FIG. 3. *Comparison of agar dilution with disc diffusion susceptibility tests for 70 Bacteroides strains against chloramphenicol.*

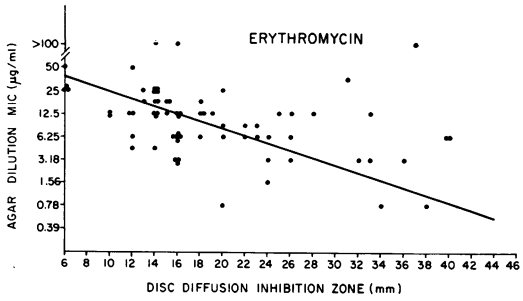

FIG. 4. *Comparison of agar dilution with disc diffusion susceptibility tests for 70 Bacteroides strains against erythromycin.*

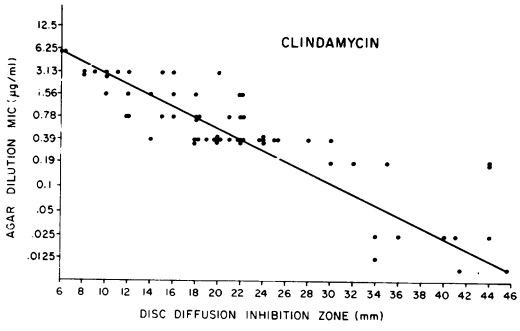

FIG. 5. *Comparison of agar dilution with disc diffusion susceptibility tests for 70 Bacteroides strains against clindamycin.*

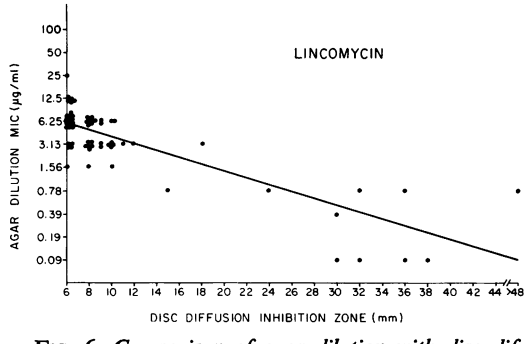

FIG. 6. *Comparison of agar dilution with disc diffusion susceptibility tests for 70 Bacteroides strains against lincomycin.*

between zone diameters and MIC values for erythromycin, clindamycin, and lincomycin. In the case of erythromycin (Fig. 4), the points are widely scattered above and below the regression line. Although the correlation was better with clindamycin (Fig. 5), several *Bacteroides* strains with zones of inhibition smaller than 16 mm (the presently accepted cut-off for "susceptibility" with the 2-μg disc) were clearly susceptible when the agar dilution technique was utilized. Finally, as shown in Fig. 6, although the majority of the strains would be called "resistant" to lincomycin by the disc diffusion technique, most of these strains were "susceptible" when the agar dilution method was employed.

DISCUSSION

Bacteroides infections represent a formidable management problem, partially because of limited in vitro and in vivo effectiveness of many currently available antibiotics against these microorganisms. In addition, there are no comparative studies of clinical efficacy of those antimicrobials recommended for the treatment of *Bacteroides* infections. This situation is further clouded by a lack of standardization of antibiotic susceptibility testing techniques for anaerobic microorganisms among various medical centers.

Most *Bacteroides* strains have been previously assumed to be susceptible to tetracycline and chloramphenicol, causing some bacteriology laboratories to forego susceptibility testing on these isolates. However, the present report confirms our previous findings, as well as those from other medical centers, showing that an impressive percentage of these organisms are tetracycline-resistant (2, 3, 6, 8). With two exceptions, the isolates we tested were susceptible to chloramphenicol. Nevertheless, reports of chloramphenicol resistance by other investigators (5), as well as the known hazards of this drug, make the availability of alternative effective agents desirable.

The results of the present study demonstrate impressive in vitro effectiveness for clindamycin, lincomycin, and carbenicillin against the majority of the *Bacteroides* strains tested. Although large doses of penicillin have been suggested as potentially effective therapy for *Bacteroides* infections, clinical results with this drug have been disappointing (2). Whether or not carbenicillin is any more effective remains to be seen. Similarly, there is only one report regarding the clinical usefulness of lincomycin or clindamycin for *Bacteroides* sepsis (9). Clindamycin appeared to be the most active drug on a weight basis, but is currently unavailable for parenteral use. Oral administration may not be suitable in the clinical settings associated with many *Bacteroides* infections (2). Lincomycin can be administered parenterally and deserves a trial in the treatment of *Bacteroides* sepsis.

Expectations for a reliable modification of the Kirby-Bauer disc diffusion technique for *Bacteroides* susceptibility testing were not borne out in the present study for many of the antibiotics used. This is in agreement with the recent findings of Thornton and Cramer (8). Disc diffusion tests for tetracycline and chloramphencol, however, were reliable for prediction of susceptibility. It is possible that clindamycin and lincomycin discs of greater potency would be helpful for bacteroides susceptibility testing by the disc diffusion method and would provide a closer correlation with broth or agar dilution techniques.

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