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Bacteriostatic and Bactericidal Activities of Various Antibiotics Against *Bacteroides fragilis*[1]

R. J. ZABRANSKY, J. A. JOHNSTON, AND K. J. HAUSER

Microbiology Research Laboratory, Department of Pathology, Mount Sinai Medical Center, Milwaukee, Wisconsin 53233

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The minimal inhibitory concentrations and minimal bactericidal concentrations of seven antibiotics were determined by broth dilution tests for 50 clinical isolates of *Bacteroides fragilis*. Chloramphenicol, erythromycin, lincomycin, clindamycin, and rifampin inhibited 80 to 100% of the strains at concentrations attainable in the serum. Penicillin and tetracycline were less effective, inhibiting 4 and 40% of the strains, respectively, at concentrations attainable in the serum. The strains exhibited a bimodal distribution with tetracycline but a very narrow pattern of susceptibility with chloramphenicol. Bactericidal concentrations were 4- to 128-fold higher than minimal inhibitory concentrations for all antibiotics; only clindamycin showed bactericidal activity at levels attainable in serum. Clindamycin was significantly more effective than lincomycin as determined by tests of inhibitory and bactericidal activity. The distinct patterns of susceptibility of *B. fragilis* may be used for the preliminary selection of antimicrobial therapy.

The recent increase of interest in clinical anaerobic bacteriology was aided by the development of the Gas-Pak anaerobic jar (2) and the special techniques developed by the Anaerobe Laboratory at the Virginia Polytechnic Institute (7). These have resulted in a number of reports illustrating the high frequency of isolation of anaerobic bacteria, particularly *Bacteroides*, from clinical specimens (11, 14, 15, 19). *B. fragilis*, an obligately anaerobic gram-negative bacillus, is recognized as a significant pathogen. This organism is not a fastidious anaerobe; it grows well on most laboratory media, and although blood does enhance its growth it is not required. *B. fragilis* can grow in the presence of 10% bile or bile plus deoxycholate (17); is resistant to certain antibiotics, especially the aminoglycosides (3); and can exist at a *p*H below 5.4 (7). Therefore, the apparent frequency of isolation, particularly in pure culture, and the relative resistance of the species indicate the need for more information on its antibiotic susceptibility.

The determination of the antibiotic susceptibility of any clinical isolate is of principal importance. At present, there is no acceptable, reproducible test for the rapid determination of the susceptibility of *B. fragilis* to antibiotics. The only reliable method is a dilution test which is expensive and time-consuming, especially for anaerobic bacteria. It is of current interest to determine whether this common isolate has a predictable susceptibility pattern. This was suggested by Finegold and associates (5), who used it as a guide for the preliminary identification of the organism.

The World Health Organization (WHO) Expert Committee in their report in 1971 (4) recommended that the broth dilution test should be the reference procedure for any antimicrobial susceptibility test. The broth dilution test is the basis for the determination of resistance or susceptibility in the widely used and standardized diffusion test described by Bauer et al. (1). In 1966, Keusch and O'Connell (9) described a dilution procedure in which 1-ml volumes of thioglycolate broth were used. The dilution scheme described by the WHO International Collaborative Study (4) uses the same volumes of broth, but the antibiotic concentrations are selected for the convenience of plotting with logs. The present study utilizes these techniques as a basis for the determination of the antibiotic activity of a variety of agents for *B. fragilis*.

MATERIALS AND METHODS

Fifty strains of *B. fragilis*, isolated from clinical specimens and identified by the criteria established by Dowell (3) and Smith and Holdeman (17), were used in this study. All organisms were maintained in a chopped-meat medium (19) at

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room temperature. Each strain was tested against seven antibiotics: penicillin, tetracycline, chloramphenicol, erythromycin, lincomycin, clindamycin, and rifampin. Antibiotic solutions in concentrations of 2,000 μg/ml were maintained as frozen stocks except for rifampin, which was prepared fresh prior to use. Thioglycolate broth containing dextrose but no indicator (THIO) was used as the broth medium for all tests.

Minimal inhibitory concentration. A 48-hr culture of the organisms, grown in THIO supplemented with 10% horse serum, was diluted 1:50 in THIO and was used as the inoculum. This inoculum represented an average of 7×10^6 organisms per ml as determined by viable plate counts. The antibiotics were diluted in THIO, and 1 ml of each antibiotic dilution plus 1 ml of inoculum were placed in tubes to obtain final antibiotic concentrations ranging from 0.008 to 128 μg/ml. The tubes were incubated at 35 C for 48 hr in a Gas-Pak system or in an anaerobic incubator, which had an atmosphere of 10% CO_2, 10% H_2, and 80% N_2. Gas-Pak catalysts were used in the anaerobic incubator. The first tube showing no turbidity was taken as the end point of the minimal inhibitory concentration (MIC).

Minimal bactericidal concentration. To determine the minimal bactericidal concentration (MBC), a calibrated loop (0.01 ml) was used to transfer the broth from each tube containing the MIC or greater of a drug to an enriched blood agar plate. After the plates were incubated as above, the end point was read as the lowest drug concentration allowing no growth.

RESULTS AND DISCUSSION

Our choice of thioglycolate broth was based upon the work of Keusch and O'Connell (9) and on its ability to promote the rapid growth of *B. fragilis*. It has been stated that reducing agents produce aberrant results in the in vitro activity of antibiotics (6). To evaluate the effect of thioglycolate on the antibiotics, Trypticase soy broth (TSB) was compared with THIO under identical conditions. MIC values in TSB and THIO were identical for chloramphenicol and tetracycline; for all other antibiotics, they were one dilution lower in TSB. MBC values in THIO and TSB were identical for chloramphenicol, one dilution lower in TSB for clindamycin, lincomycin, erythromycin, and tetracycline, and two dilutions lower in TSB for rifampin and penicillin.

Much of the earlier information on the susceptibility of *Bacteroides* was determined by diffusion studies, but the technique of Bauer et al. (1) for susceptibility testing of aerobic bacteria was not designed for fastidious or anaerobic bacteria. Similarly, the work of the WHO Expert Committee (4) provided information on susceptibility tests performed only under aerobic conditions. Recent papers have shown that reliable results may be obtained by the diffusion method with anaerobic bacteria, but not all of these reports contain information on the speciation of these organisms. Table 1 shows the number and the cumulative percentage of strains of *B. fragilis* inhibited and killed at specific concentrations of the antibiotics tested.

Of all the antibiotics tested, penicillin was the least effective against *B. fragilis*. Only 4% of the isolates were inhibited and none was killed by 2 μg/ml, a level easily attainable in serum (16). The MBC levels of penicillin were four- to eightfold higher than the MIC levels. The data presented here and by Nastro and Finegold (13), Martin, Gardner, and Washington (12), and others show that *B. fragilis* is indeed resistant to concentrations of penicillin attainable in serum (16). In fact, levels in excess of 128 μg/ml may be needed to kill the organisms. Martin et al. (12) reported that 11 of 25 strains were not killed by penicillin concentrations of 100 μg or more/ml. The inhibitory effects of penicillin on our 50 isolates were essentially the same as those observed by Martin et al. (12) and Kislak (10), both of whom used agar dilution techniques.

Chloramphenicol was very active against *B. fragilis* at concentrations attainable in serum. This antibiotic has been one of the drugs of choice for anaerobic infections, perhaps because of the apparently uniform susceptibility of *Bacteroides*. Chloramphenicol inhibited all strains at a concentration of 16 μg/ml and inhibited a majority of them at 8 μg/ml. These concentrations may be achieved in serum with normal dosages (16). No isolates were inhibited by less than 4 μg/ml. The bacteriostatic nature of chloramphenicol is shown by the 32-fold or greater difference in the MBC and MIC levels.

Tetracycline has also been a primary drug of choice in anaerobic infections for many years. Several investigators (8) have reported a high degree of susceptibility of *B. fragilis* to this agent, but recent studies (18) have indicated increased resistance. The inhibitory levels of tetracycline resulted in a typical bimodal pattern also described for other organisms with this antibiotic (Table 1). Data available from other studies indicate that this is a constant phenomenon and is also demonstrable with analogues of tetracycline (10). Tetracycline inhibited 40% of our strains at concentrations attainable in serum (16). MBC levels of tetracycline were consistently 64-fold higher than the MIC levels and might have exhibited the same bimodal distribution if concentrations in excess of 128

TABLE 1. *Number and cumulative percentage of strains of Bacteroides fragilis inhibited and killed by various drug concentrations*

Drug	Effect	Drug concn (μg/ml)[a]														
		0.015	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Penicillin	Inhibited															
	No.	—	—	—	—	—	—	—	2	0	4	16	25	1	1	1
	Percent	—	—	—	—	—	—	—	4	4	12	44	94	96	98	100
	Killed															
	No.	—	—	—	—	—	—	—	—	—	—	2	10	12	8	18
	Percent	—	—	—	—	—	—	—	—	—	—	4	24	48	64	100
Chloramphenicol	Inhibited															
	No.	—	—	—	—	—	—	—	—	5	39	6				
	Percent	—	—	—	—	—	—	—	—	10	88	100				
	Killed															
	No.	—	—	—	—	—	—	—	—	—	—	—	2	1	3	44
	Percent	—	—	—	—	—	—	—	—	—	—	—	4	6	12	100
Tetracycline	Inhibited															
	No.	—	—	—	—	1	5	10	4	2	—	—	12	15	1	
	Percent	—	—	—	—	2	12	32	40	44	44	44	68	98	100	
	Killed															
	No.	—	—	—	—	—	—	—	—	—	—	1	3	5	7	34
	Percent	—	—	—	—	—	—	—	—	—	—	2	8	18	32	100
Rifampin	Inhibited															
	No.	1	9	13	10	12	3	1	—	—	—	1				
	Percent	2	20	46	66	90	96	98	98	98	98	100				
	Killed															
	No.	—	—	—	—	—	—	2	3	6	14	9	9	1	2	4
	Percent	—	—	—	—	—	—	4	10	22	50	68	86	88	92	100
Erythromycin	Inhibited															
	No.	—	—	—	3	2	1	6	22	12	1	1	—	—	—	2
	Percent	—	—	—	6	10	12	24	68	92	94	96	96	96	96	100
	Killed															
	No.	—	—	—	—	—	—	—	2	—	6	7	10	4	8	13
	Percent	—	—	—	—	—	—	—	4	4	16	30	50	58	74	100
Lincomycin	Inhibited															
	No.	—	—	—	—	—	—	2	19	19	9	1				
	Percent	—	—	—	—	—	—	4	42	80	98	100				
	Killed															
	No.	—	—	—	—	—	—	—	—	2	3	4	10	8	8	15
	Percent	—	—	—	—	—	—	—	—	4	10	18	38	54	70	100
Clindamycin	Inhibited															
	No.	—	—	2	9	10	18	8	3							
	Percent	—	—	4	22	42	78	94	100							
	Killed															
	No.	—	—	—	1	2	2	12	5	11	11	3	3			
	Percent	—	—	—	2	6	10	34	44	66	88	94	100			

[a] At the lowest concentration tested, 0.008 μg/ml, none of the drugs inhibited or killed any of the strains.

μg/ml had been tested. Nastro and Finegold (13), using fewer than 10 colonies as their criterion for MBC determination, reported that 2 of the 19 strains they tested were killed by less than 10 μg of tetracycline/ml. Using complete killing as the MBC interpretation, Martin et al. (12) reported that 3 of 25 isolates were killed by 6.2 μg or less of tetracycline/ml. Our finding that no isolates were killed by 8 μg or less/ml confirmed the lack of bactericidal activity of tetracycline.

A new antibiotic used primarily for treatment of tuberculosis, rifampin, was very active against our strains of *B. fragilis*, inhibiting all but one strain at 1 μg or less/ml (Table 1). All of 195 isolates tested by Martin and associates (12) were inhibited by 1.6 μg of rifampin/ml; Nastro and Finegold (13) and Kislak (10) reported similar results. The bactericidal activity of rifampin was poor, as bactericidal levels averaged 128 times the inhibitory levels.

Two agents primarily active against gram-positive bacteria, erythromycin and lincomycin, displayed good in vitro inhibitory activity against *B. fragilis* (Table 1), confirming the data of Kislak (10), Martin et al. (12), and Thornton and Cramer (18). Erythromycin exhibited its bacteriostatic nature by inhibiting 92% of

our strains at concentrations achievable in serum (4 μg/ml) but killing only 4% of the strains at the same concentrations (16). Levels in excess of 128 μg/ml would be needed to kill these isolates of *B. fragilis*. The activity of lincomycin was similar to that of erythromycin; 98% of the isolates were inhibited by 8 μg/ml, but levels in excess of 128 μg/ml were required to kill the same strains. These levels are now attainable in serum with higher doses of lincomycin (16).

The lincomycin data are in sharp contrast to results obtained with clindamycin, an antibiotic also originally assigned to a narrow spectrum of gram-positive activity. We found (Table 1) that clindamycin is actually bactericidal to *B. fragilis* at concentrations achievable in serum. All strains were inhibited by 2 μg or less of clindamycin/ml and, although the bactericidal concentrations were four- to eightfold higher, 88% of the strains were killed by 8 μg/ml. As with lincomycin, these levels are attainable in serum with higher doses of clindamycin. Among the antibiotics we tested, the activity of clindamycin was the greatest. This is not in agreement with other reports. Using the same criterion as we did for the interpretation of the MBC levels, Nastro and Finegold (13) reported only 1 or 2 of 19 strains killed at 6.25 μg/ml, and Martin et al. (12) reported 12 of 25 strains of *B. fragilis* killed at similar levels. The inhibitory effect of clindamycin in our study was essentially the same as that reported by Martin et al. (12) and Kislak (10).

This study has shown that *B. fragilis* is inhibited in vitro by a large group of antibiotics. Of special interest is the high activity of clindamycin for *B. fragilis*. Clindamycin, like chloramphenicol, inhibited all strains of *B. fragilis* at low concentrations. With the eventual availability of clindamycin in parenteral form, we will have another antibiotic for the treatment of these infections. Its bactericidal nature and lack of potential toxicity are two distinct advantages for its use in the treatment of anaerobic infections, especially when host defenses are impaired. Preliminary results from clinical trials of clindamycin indicate that it will probably be one of the drugs of choice for anaerobic infections.

In that *B. fragilis* does exhibit some distinct patterns of susceptibility with certain antibiotics, susceptibility information may be inferred from the species identification of the organism and an empirical choice of the antibiotic may be made. Because of the lack of reliable diffusion methods for anaerobes and the expense and time required for broth and agar dilution studies, data such as these may be useful in the selection of antibiotic therapy. This would be of particular importance in the treatment of anaerobic infections caused by *B. fragilis*, the most common and most resistant of the anaerobic gram-negative bacteria. Projections of this type should not take the place of a properly performed dilution susceptibility test which could be indicated under a variety of clinical circumstances.

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