

# Standardized Antimicrobial Disc Susceptibility Testing of Anaerobic Bacteria

## I. Susceptibility of *Bacteroides fragilis* to Tetracycline

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A modified Bauer-Kirby-Sherris-Turck method for disc susceptibility testing of anaerobic bacteria is presented. When tetracycline was used against 100 strains of *Bacteroides fragilis* as a model, reasonably reproducible results were obtained after overnight incubation in both the GasPak atmosphere and an atmosphere achieved by adding 10% CO<sub>2</sub> to a mixture of 10% H<sub>2</sub> and 90% N<sub>2</sub>. The minimal inhibitory concentration for the strains determined by the agar dilution technique correlated well with the results of disc tests performed in the GasPak atmosphere with 30- $\mu$ g tetracycline discs. Among 63 strains isolated from 1970 to the present, only 24 (38.1%) were found to be susceptible to tetracycline.

With the increasing awareness of the importance of anaerobic bacteria in infectious processes, the development of a simple, rapid and reproducible method for determining the susceptibility of these organisms to antimicrobial agents becomes important. Although many anaerobic bacteria have predictable patterns of susceptibility to these agents and clinicians have relied on these as a guide to therapy (8), there are often exceptions to the general patterns. As examples, penicillin G is considered the drug of choice for infections due to *Clostridium* species, but occasional strains are resistant to penicillin G; tetracycline has been considered a drug of choice for infections due to *Bacteroides fragilis*, but resistant strains of *Bacteroides* species and *B. fragilis* are now being found (3, 11). Determination of the susceptibility of individual isolates to various antimicrobial agents would provide more specific information to the clinician for guidance of therapy.

There is a great degree of variability in methods now in use for determining antimicrobial susceptibility of anaerobic bacteria. Investigators have used a variety of broth or agar dilution and paper disc techniques (2, 6, 9, 10). Results vary considerably among the various studies, with variability probably due to differences in media used, age and size of inoculum, and method and length of anaerobic

incubation. Little has been done to standardize methodology and to correlate results of paper disc tests with those of broth or agar dilution tests. A number of laboratories use the method and standards of Bauer et al. (1), although this has not been standardized for anaerobic conditions and erroneous interpretations can be made.

The method of Bauer et al. (1) has been found acceptable for aerobic and facultative bacteria and has recently been recommended by the Food and Drug Administration for adoption as the single recognized method for antibiotic disc susceptibility testing in this country (5). The Food and Drug Administration recommended that susceptibility of *Bacteroides* species and other anaerobes be determined by broth or agar dilution methods but did not specify methods or conditions to be used.

We have believed for some time that a standardized disc test for anaerobic bacteria with interpretive standards similar to those of Bauer et al. (1) would be of great value to diagnostic microbiology laboratories, and we have been directing efforts toward developing such a method. Our primary aims have been to select parameters of media, age and size of inoculum, and time of incubation which give satisfactory disc test results after overnight incubation with rapidly growing strains, and to corre-

late these results with those obtained by a dilution method with similarly selected parameters. Since *B. fragilis* is one of the most frequent anaerobic pathogens and often the most difficult to treat, we considered it an important organism with which to initiate our studies. Additionally, tetracycline was selected for the initial studies because resistance of *B. fragilis* to this drug is being reported.

The purpose of this report is to present the methods selected and to analyze the results obtained by use of tetracycline against 100 strains of *B. fragilis* as a preliminary model for further work toward standardization of susceptibility testing of anaerobes. Subsequent studies will include work with other rapid-growing and slow-growing anaerobes as well.

### MATERIALS AND METHODS

**Preliminary studies.** Thirty-five strains of anaerobic bacteria representing the genera *Bacteroides*, *Fusobacterium*, *Vibrio*, *Veillonella*, *Peptococcus*, *Peptostreptococcus*, *Bifidobacterium*, and *Clostridium* were used in tests for selection of media to be used. Commercially available fluid media [fluid thioglycollate medium (BBL), Schaedler broth (BBL), and thioglycollate medium without indicator—135C (BBL)] with and without additives such as ascitic fluid, dithiothreitol, hemin, menadione, and  $\text{NaHCO}_3$ , alone and in combination, were surveyed. Thioglycollate medium without indicator—135C to which was added 5  $\mu\text{g}$  hemin per ml prior to autoclaving and 1 mg of  $\text{NaHCO}_3$ /ml plus 0.5  $\mu\text{g}$  of menadione/ml (filter-sterilized) after autoclaving, as suggested by Sawyer et al. (12), was chosen as the medium for growing the inoculum. It was found to give good growth of the rapid-growing strains such as *Bacteroides fragilis*, *Clostridium perfringens* and *Fusobacterium varium* within 4 to 6 hr of incubation and of the slower-growing strains such as *B. melaninogenicus*, *B. oralis*, and *Peptostreptococcus* species after overnight incubation. This medium is henceforth referred to as THCM. Fluid thioglycollate medium with hemin and 25% ascitic fluid was equally good for promoting growth but has the disadvantages that ascitic fluid is extremely variable in composition, expensive, and difficult to obtain, and that excessive turbidity which does not represent viable cells is present after growth of some bacteria.

The same strains were tested on several commercially available agar bases [Brucella (Pfizer Co., Inc.) Columbia (BBL), Infusion (BBL), Mueller-Hinton (BBL), Mueller-Hinton (Difco), Schaedler (BBL), and Trypticase soy (BBL)] with and without various additives. Brucella agar with 5% sheep blood and 0.5  $\mu\text{g}$  of menadione per ml was selected as the medium for the disc diffusion test. This medium supported the growth of rapid-growing strains so that tests could be read after overnight incubation. Although many anaerobic bacteria were able to grow on Brucella agar without additives, a number of them either required or were stimulated by hemin or blood,

and one strain of *B. melaninogenicus* required both blood and menadione for growth on solid medium. Therefore, we believed that these ingredients should be added to the test agar to provide a medium on which most anaerobic bacteria would grow and which could be used in future work with anaerobes more fastidious than *B. fragilis*. Schaedler and Columbia agars supplemented with blood and menadione were equally good for growth of the organisms. Schaedler agar was not selected because of its high glucose content. The choice between Columbia and Brucella agars was arbitrary. Infusion agar with blood and menadione supported growth of all bacteria tested, but growth was not as good on this medium as on the above three media. Mueller-Hinton agar with blood and menadione yielded no growth after 18 to 24 hr with three of the strains tested, and Trypticase soy agar with blood and menadione yielded no growth after 18 to 24 hr with two of the strains tested.

Fifteen strains of *B. fragilis* were tested for their susceptibility to tetracycline by both broth and agar dilution methods. Twofold dilutions of tetracycline were made in Brucella broth and agar to which menadione and laked sheep blood were added so that their final concentrations in the test were 0.5  $\mu\text{g}/\text{ml}$  and 5%, respectively. The blood was laked by freezing and thawing. An inoculum of 0.5 ml of a 6-hr culture in THCM diluted in Brucella broth to just beyond visible turbidity ( $10^5$  to  $10^6$  viable organisms/ml) was added to 0.5 ml of the broth containing tetracycline. The agar plates were inoculated with  $10^4$  to  $10^5$  viable cells by means of a replicating device (13) with the inoculum in the wells adjusted to the #1 MacFarland nephelometer standard. Both sets of tests were read after 18 to 24 and 42 to 48 hr of incubation at 37 C in a modified Brewer jar made anaerobic by evacuating and filling with oxygen-free gas five times. The final atmosphere was achieved by adding 10%  $\text{CO}_2$  to a mixture of 10%  $\text{H}_2$  and 90%  $\text{N}_2$ . This atmosphere will be referred to as 10%  $\text{H}_2$ , 90%  $\text{N}_2$  + 10%  $\text{CO}_2$ . The jar contained palladium-coated alumina catalyst. The minimal inhibitory concentration (MIC) in each test was recorded as the highest dilution showing no macroscopically visible growth. MIC values with both tests were usually the same, and were not more than one dilution step different in instances where variation occurred. With both tests, the MIC was frequently one dilution step lower at 42 to 48 hr. The agar dilution test was easier to read and interpret at both incubation periods. Because it is more economical to perform the agar dilution method if one is testing many strains and because results are easier to read, the agar dilution method for determining the MIC was chosen for the remainder of this study.

Laked blood had been used in the studies above to allow observation of growth in the broth dilution tests. To determine how whole blood or laked blood would affect results in the agar dilution method, the MIC for 31 strains of *B. fragilis* was determined as described above with the use of whole or laked blood in two sets of plates. At 42 to 48 hr, the MIC values for seven of the strains differed by one dilution step,

with values higher more often with whole blood than with laked blood. Results on laked blood-agar were easier to read because growth caused a discoloration of the blood underneath the growth and in the area surrounding the growth, which made the decision of growth or no growth less difficult in several instances.

A similar experiment was performed with tetracycline discs, 30  $\mu\text{g}$  (BBL), applied to plates swabbed with 10 strains of *B. fragilis* grown for 4 to 6 hr in THCM, diluted to the density of one-half the turbidity of the #1 MacFarland standard, as in the method of Bauer et al. (1). At 18 to 24 and 42 to 48 hr, inhibition zone diameters were often the same or not more than 2 mm larger or smaller for each strain on the two types of blood-agar if the zone of inhibition was measured by reflected light on the surface of the plate. Discoloration caused by growth on laked blood diffused into the zone of inhibition, and if plates were read by transmitted light an erroneously small diameter would be measured. Accordingly, it was decided that whole blood would be used for the disc test.

**Antimicrobial susceptibility tests: bacterial strains.** Susceptibility tests were performed on 100 strains of *B. fragilis*. Fifteen strains had been isolated from 1954 to 1959, 22 from 1960 to 1969, and the remainder from 1970 to the present time. Most of them had been isolated initially on nonselective media; a few had been received from other laboratories, and information regarding initial isolation medium was not available. Eighty-three were from clinical specimens, and 17 were from feces. They were identified by methods previously described (14).

**Paper-disc susceptibility test.** The inoculum was prepared by heavily inoculating each strain into THCM (three to four colonies or a 3-mm loopful). The medium was prepared fresh each week, steamed for 10 min, and cooled prior to the addition of menadione and  $\text{NaHCO}_3$ . Cultures were incubated at 37 C for 4 to 6 hr and adjusted to one-half the density of the #1 MacFarland standard (1). The inoculum was applied by swabbing onto duplicate plates of Brucella agar containing 5% defibrinated sheep blood and 0.5  $\mu\text{g}$  of menadione per ml, freshly prepared and dried at 37 C for 45 to 60 min prior to use or incubated overnight for use the next day. The agar was 5 to 6 mm in depth and was at pH 7.4 prior to use. Tetracycline discs, 5 and 30  $\mu\text{g}$ , were applied, and one set of plates was incubated under 10%  $\text{H}_2$ , 90%  $\text{N}_2$  + 10%  $\text{CO}_2$  as described above; the other set was incubated in a GasPak jar. Catalysts for both anaerobic systems were reactivated after each use by heating to 160 to 170 C for 1.5 to 2 hr. Except for the time required for setting up the tests and the time required to achieve anaerobiosis, no prediffusion period was used. Usually only 12 strains were done at one time, and both jars were processed rapidly and placed in the incubator. Indicators (Disposable Anaerobic Indicator, BBL) used in the jars usually showed that conditions were completely anaerobic after 2 to 3 hr. Zones of complete inhibition of growth around the discs were measured with calipers after 18 to 24 and 42 to 48 hr.

One strain (B-1) was included each day that tests were performed. This strain was also used in 50 additional determinations on 3 different days with both 5- and 30- $\mu\text{g}$  discs to determine reproducibility of results.

**Agar dilution tests.** Agar dilution tests were set up as described above in preliminary studies. Tests were incubated in an atmosphere of 10%  $\text{H}_2$ , 90%  $\text{N}_2$  + 10%  $\text{CO}_2$ , or in a GasPak jar. Strain B-1 was included with each day's tests to assess reproducibility of results. It was tested six times.

## RESULTS

Inhibition zone diameters around the tetracycline discs were as easily measured at 18 to 24 hr as they were at 42 to 48 hr. The diameters with the majority of strains varied only 1 to 2 mm at the two incubation periods in each anaerobic system. Exceptions to this were as follows: in the 10%  $\text{H}_2$ , 90%  $\text{N}_2$  + 10%  $\text{CO}_2$  atmosphere, there were 28 strains which had 3 to 9 mm larger or smaller zones at 42 to 48 hr around the 30- $\mu\text{g}$  disc and 12 strains with variations of 3 to 8 mm around the 5- $\mu\text{g}$  disc; in the GasPak atmosphere, there were 15 strains with a variation of 3 to 7 mm around the 30- $\mu\text{g}$  disc and 10 strains with a variation of 3 to 5 mm around the 5- $\mu\text{g}$  disc. Three strains showed this variation with discs of both strengths in both atmospheres. An unexpected observation with regard to variations in zone diameters was that with sensitive strains the zones usually became larger and with resistant strains they became smaller with longer incubation.

In general, the diameters of the zones of inhibition tended to be slightly larger in the  $\text{H}_2$ ,  $\text{N}_2$  +  $\text{CO}_2$  atmosphere than in the GasPak atmosphere at both 18 to 24 and 42 to 48 hr. The 5- $\mu\text{g}$  discs produced zones of inhibition from 5 to 11 mm smaller than did the 30- $\mu\text{g}$  discs tested against the same strains in the  $\text{H}_2$ ,  $\text{N}_2$  +  $\text{CO}_2$  atmosphere and from 3 to 11 mm smaller than did the 30- $\mu\text{g}$  discs in the GasPak atmosphere. The distribution of zone diameters with 5- and 30- $\mu\text{g}$  discs in the two atmospheres and for the two periods of incubation followed bimodal curves as illustrated in Fig. 1 and 2. Most strains appear to fall into either a resistant or susceptible group with a few strains in an intermediate group.

The diameters of zones of inhibition around the 5- and 30- $\mu\text{g}$  discs at both 18 to 24 hr and 42 to 48 hr were plotted against a log of the MIC read at the same interval in each anaerobic system for each of the 100 strains. Regression lines were calculated by the method of least squares (4) and were drawn to demon-

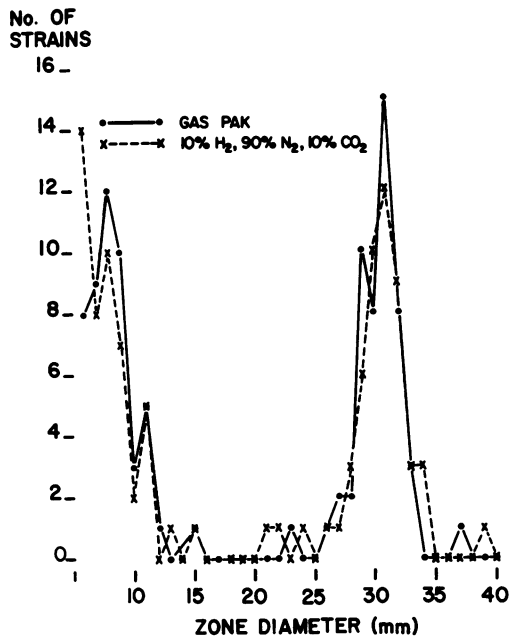


FIG. 1. Distribution of inhibition zone diameters around tetracycline discs, 5 µg, 18 to 24 hr.

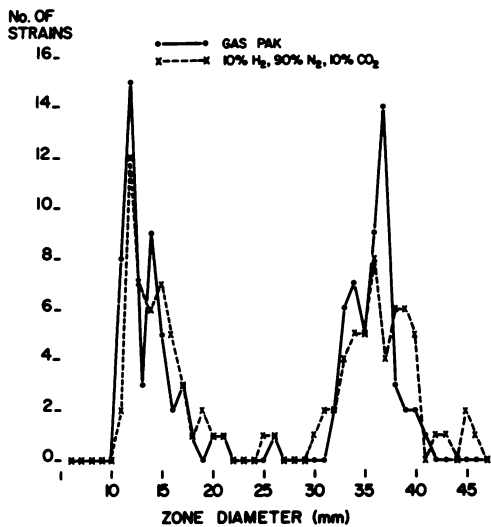


FIG. 2. Distribution of inhibition zone diameters around tetracycline discs, 30 µg, 18 to 24 hr.

strate the relationship between zone diameter and MIC data. Figure 3 illustrates the relationship observed between the MIC and zones of inhibition around the 30-µg disc in the GasPak system at the two time intervals. If we define susceptible strains as those having an MIC  $\leq 1.6$  µg/ml, resistant strains as  $\geq 12.5$  µg/ml and intermediate strains as 3.1 to 6.2

µg/ml, then correlation at 18 to 24 hr is poor. Strains with zone diameters of  $\leq 11$  mm would be considered resistant; 12 to 25 mm, intermediate; and  $> 25$  mm, susceptible. As Fig. 3A indicates, there were 18 strains with an MIC of  $\geq 12.5$  µg/ml and 1 strain with an MIC of 0.2 µg/ml which had zone diameters in the intermediate range and one with an MIC of 3.1 µg/ml with a zone diameter in the susceptible range. At 48 hr, correlation was better, as indicated in Fig. 3B. Zone diameters were inter-

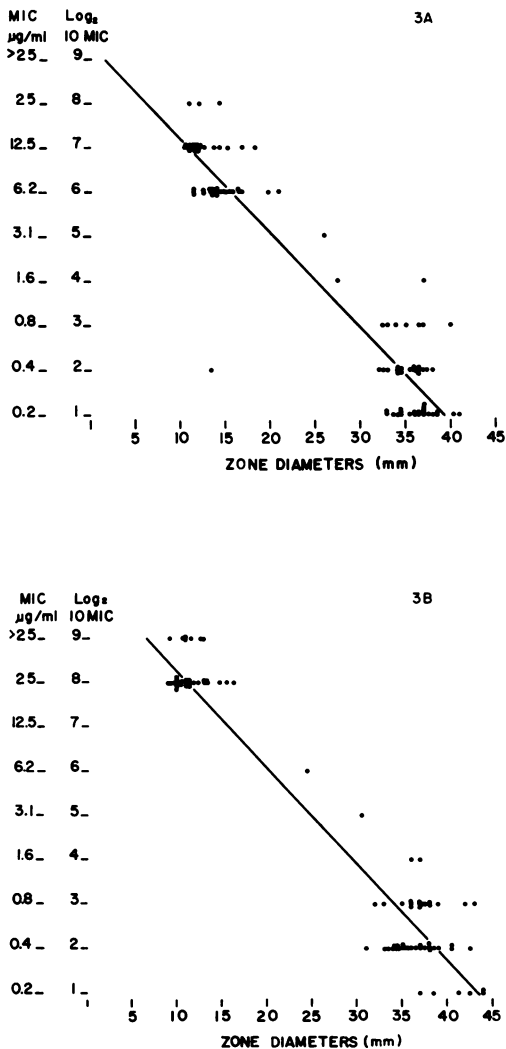


FIG. 3. Relationship of inhibition zone diameters around 30-µg tetracycline discs and MIC values in the GasPak atmosphere. (A) Determinations made at 18 to 24 hr. Least squares line for  $y = a + bx$ ;  $b = -0.211$ ,  $a = 9.285$ . (B) Determinations made at 42 to 48 hr. Least squares line for  $y = a + bx$ ;  $b = -0.216$ ,  $a = 10.368$ .

preted as follows:  $\leq 16$  mm, resistant; 17 to 29 mm, intermediate;  $\geq 30$  mm, susceptible. Only one strain with intermediate susceptibility by the agar dilution test fell into the susceptible category by disc test.

MIC results were often difficult to interpret at 18 to 24 hr, and the 42 to 48 hr reading was considered technically better, but there was no difficulty in measuring zone diameters at 18 to 24 hr. The relationship of zone diameters at 18 to 24 hr and MIC values at 42 to 48 hr was then plotted, and regression lines were calculated for each disc strength and each condition of incubation. Figure 4 shows the relationship between zone diameters with the 5- and 30- $\mu\text{g}$  discs and MIC values in the GasPak atmosphere. Correlation was slightly better with the 5- $\mu\text{g}$  than with the 30- $\mu\text{g}$  discs. Figure 5 illustrates the relationship of results of the two tests in the 10%  $\text{H}_2$ , 90%  $\text{N}_2$  + 10%  $\text{CO}_2$  atmosphere. Correlation was about the same for discs of both concentrations.

Table 1 indicates tentative interpretation of zone diameters based on data derived from the distribution of zone diameters and regression line analysis of zone diameters measured at 18 to 24 hr and MIC values at 42 to 48 hr. The criteria for susceptibility are based on tetracycline concentrations in the blood achieved with ordinary dosage schedules (levels of 1 to 2  $\mu\text{g}/\text{ml}$  are commonly achieved with oral dosage and of 5 to 10  $\mu\text{g}/\text{ml}$  with intravenous administration).

Results of tests to determine reproducibility of tetracycline zone diameters with strain B-1 are shown in Table 2. In 56 tests with 5- $\mu\text{g}$  tetracycline discs performed on 9 different days, the zone diameters in both atmospheres varied over a range of 10 mm. In 56 tests with 30- $\mu\text{g}$  tetracycline discs performed on 10 different days, the zone diameters varied over a range of 7 mm in the GasPak atmosphere and 8 mm in the 10%  $\text{H}_2$ , 90%  $\text{N}_2$  + 10%  $\text{CO}_2$  atmosphere.

Table 3 illustrates an analysis of variance of results due to disc potency and gaseous atmosphere at the 95% confidence level. When variances with 5- $\mu\text{g}$  tetracycline discs were analyzed, there was no significant difference between the two atmospheres. With 30- $\mu\text{g}$  discs, the variance was significantly different. Variances between 5- and 30- $\mu\text{g}$  tetracycline discs in the GasPak atmosphere were significantly different.

In the tests to determine reproducibility of the agar dilution method with strain B-1, the MIC was 0.4  $\mu\text{g}/\text{ml}$  on all but one occasion. One determination in the 10%  $\text{H}_2$ , 90%  $\text{N}_2$  +

10%  $\text{CO}_2$  atmosphere resulted in an MIC of 0.8  $\mu\text{g}/\text{ml}$ .

## DISCUSSION

Most of the recent effort in antimicrobial susceptibility testing has been directed toward the development of a standard reference method (7) and toward the recommendation of a single standardized disc method for susceptibility testing of rapidly growing aerobic and facultative pathogens (5). Similar efforts are

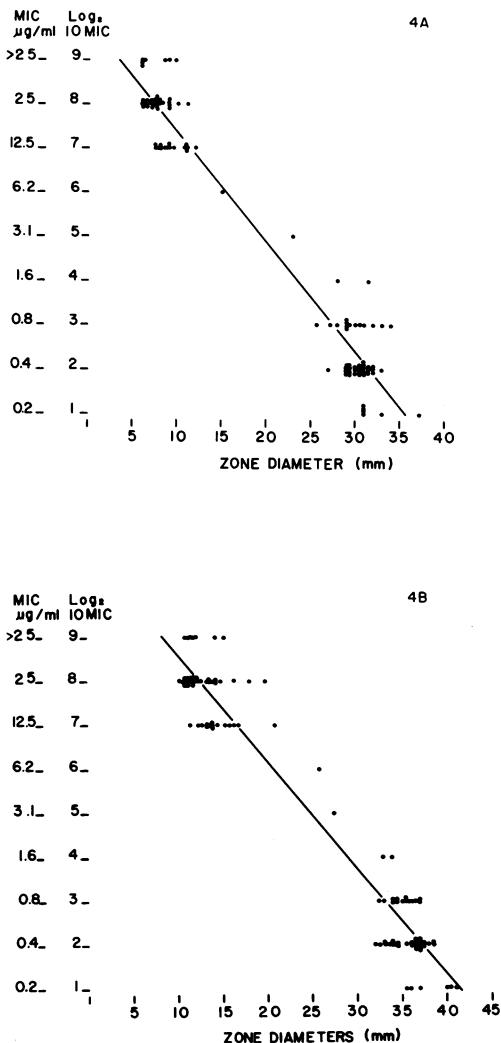


FIG. 4. Relationship of zone diameters measured at 18 to 24 hr and MIC values read at 42 to 48 hr in the GasPak atmosphere. (A) Tetracycline discs, 5  $\mu\text{g}$ . Least squares line for  $y = a + bx$ ;  $b = -0.248$ ,  $a = 9.827$ . (B) Tetracycline discs, 30  $\mu\text{g}$ . Least squares line for  $y = a + bx$ ;  $b = -0.245$ ,  $a = 11.101$ .

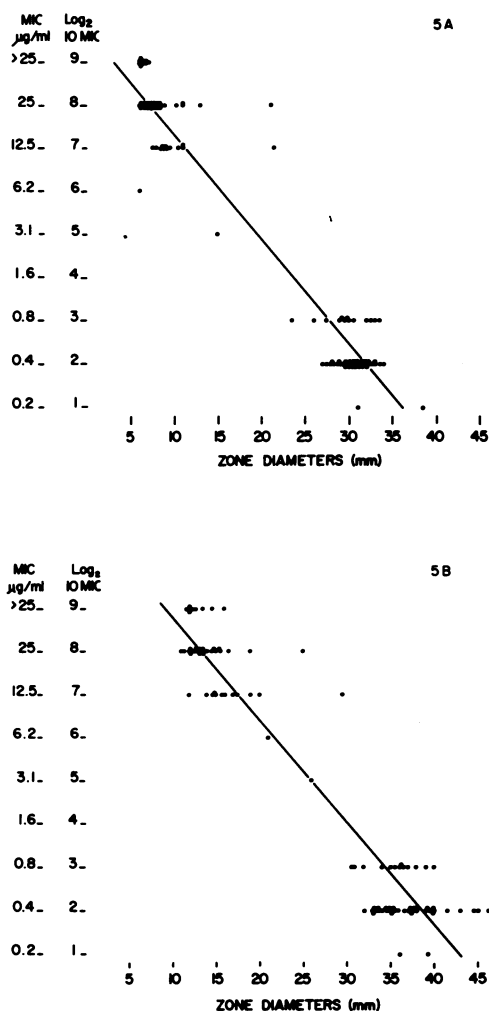


FIG. 5. Relationship of zone diameters measured at 18 to 24 hr and MIC values read at 42 to 48 hr in 10%  $H_2$ , 90%  $N_2$  + 10%  $CO_2$  atmosphere. (A) Tetracycline disc, 5  $\mu$ g. Least squares line for  $y = a + bx$ ;  $b = -0.245$ ,  $a = 9.800$ . (B) Tetracycline disc, 30  $\mu$ g. Least squares line for  $y = a + bx$ ;  $b = -0.239$ ,  $a = 11.255$ .

needed for susceptibility testing of anaerobic pathogens.

Development of resistance to antimicrobial agents has long been recognized as a problem among aerobic and facultative organisms. A similar situation undoubtedly exists among most anaerobic pathogens. An example of this is the emerging resistance of *B. fragilis* to tetracycline. In 1966, Keusch and O'Connell (11) reported only four of nine strains of *Bacteroides* tested to be susceptible. In 1970, Bodner et al. (3) reported that 18 of 38 strains tested

TABLE 1. Tentative zone diameter interpretation (18- to 24-hr incubation)

Tetracycline disc potency ( $\mu$ g)	Atmosphere	Inhibition zone diameter (mm)		
		Resistant <sup>a</sup>	Intermediate	Susceptible <sup>b</sup>
5	GasPak	$\leq 12$	13-24	$\geq 25$
5	$H_2, N_2 + CO_2$	$\leq 12$	13-24	$\geq 25$
30	GasPak	$\leq 17$	18-29	$\geq 30$
30	$H_2, N_2 + CO_2$	$\leq 22$	23-30	$\geq 31$

<sup>a</sup> Bacteria resistant to 12.5  $\mu$ g/ml or more.

<sup>b</sup> Bacteria susceptible to 1.6  $\mu$ g/ml or less.

were susceptible. More recently, 45.5% of strains of *B. fragilis* isolated in St. Louis (A. C. Sonnenwirth, *personal communication*) and 34.5% of strains of *B. fragilis* isolated at Mayo Clinic (W. J. Martin, *personal communication*) have been found susceptible. Among the strains tested in the present study, 14 of 15 isolated prior to 1960, 12 of 22 isolated from 1960 to 1969, and only 24 of 63 (38.1%) isolated from 1970 to the present were susceptible.

Our data with tetracycline against 100 strains of *B. fragilis* indicate that a modification of the method of Bauer et al. (1) results in a reasonably reproducible method for disc susceptibility testing of anaerobes. With the variables of gaseous atmosphere, incubation time, and disc potency tested, the system utilizing the GasPak, overnight incubation, and 5- $\mu$ g discs gave somewhat better correlation between MIC at 42 to 48 hr and zone diameter data. Based on MIC values and regression line analysis, there were no predictions of susceptibility or resistance by inhibition zone diameters which did not correlate with MIC values. With 30- $\mu$ g discs, under the same conditions, three strains resistant by MIC data were predicted to be intermediate by zone diameters. A similar analysis of the data obtained in the 10%  $H_2$ , 90%  $N_2$  + 10%  $CO_2$  atmosphere indicated that, at 18 to 24 hr, zone diameters with 5- $\mu$ g discs resulted in five predictions which did not correlate with MIC data, and zone diameters with 30- $\mu$ g discs resulted in two predictions which did not correlate.

Tests for reproducibility of zone diameters with strain B-1 showed greater variability with 5- $\mu$ g discs. An analysis of variance of results with the two disc potencies in the two different atmospheres indicated the least variation with 30- $\mu$ g discs in the GasPak atmosphere. Additionally, the GasPak atmosphere with activated catalyst gave fewer predictions which did not correlate with MIC data in the

TABLE 2. Variability of zone diameters

Tetracycline disc potency ( $\mu\text{g}$ )	Atmosphere	Inhibition zone diameters (mm) <sup>a</sup>				
		Minimum-maximum	Range	Mean	Mode	SD
5	GasPak	26-36	10	32	33	2.33
5	H <sub>2</sub> , N <sub>2</sub> + CO <sub>2</sub>	27-37	10	33	35	1.97
30	GasPak	35-42	7	38	38	1.41
30	H <sub>2</sub> , N <sub>2</sub> + CO <sub>2</sub>	35-43	8	39	39	2.03

<sup>a</sup> Based on 56 determinations for each set of conditions.

TABLE 3. Significance of variances between materials and methods

Tetracycline disc potency ( $\mu\text{g}$ )	Atmosphere	Mean squares deviation	F ratio <sup>a</sup>
5	GasPak	5.43	1.39
5	H <sub>2</sub> , N <sub>2</sub> + CO <sub>2</sub>	3.88	
30	GasPak	1.99	2.07
30	H <sub>2</sub> , N <sub>2</sub> + CO <sub>2</sub>	4.12	
5	GasPak	5.43	2.73
30	GasPak	1.99	

<sup>a</sup> At the 95% level of confidence, the confidence intervals are  $0.57 < F < 1.52$ .

present study, and, because of its simplicity, this atmosphere is probably the one least subject to variation in a diagnostic microbiology laboratory. Therefore, it has been selected for future studies of susceptibility testing in this laboratory.

Further work is in progress with other drugs against *B. fragilis*. We also plan to study other anaerobes and other drugs in this manner. Standards for interpretation of inhibition zone diameters applicable to all anaerobes tested will then be developed.

Conditions inherent in anaerobic incubation (anaerobiosis and CO<sub>2</sub>, with consequent lowering of pH), as well as addition of blood or other substances to culture media, are known to affect the activity of various antimicrobial agents, but are necessary for the growth of many anaerobes. Our approach has been to standardize conditions as carefully as possible and to determine the variability of results under these conditions. Once this is known for each antimicrobial agent to be considered, then the effect of varying conditions or medium composition, or both, on the activity of antimicrobial agents can be determined.

The validity of test results and their interpretation can be ascertained only when correlative clinical data are available.

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