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In Vitro Antimicrobial Susceptibility of Anaerobic Bacteria Isolated from Clinical Specimens[1]

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The minimal inhibitory concentrations of 601 clinical isolates of anaerobic bacteria to 10 different antimicrobial agents were determined by an agar-dilution technique. Nearly all strains were resistant to kanamycin and gentamicin, although moderate activity to both drugs was noted with *Fusobacterium* sp., anaerobic cocci, some strains of *Bacteroides melaninogenicus*, and nonsporeforming gram-positive bacilli. Chloramphenicol at 12.5 μg/ml inhibited all but three of the strains tested. Tetracycline at 6.25 μg/ml had high activity against all groups tested, with the exception that only 39% of strains of *Bacteroides fragilis* were inhibited at this concentration. Excluding certain species of *Bacteroides*, the majority of anaerobes were inhibited by penicillin at 3.1 μg/ml or less and by cephalothin at 12.5 μg/ml or less. Lincomycin at 6.2 μg/ml or less was active against nearly all strains. Erythromycin at a concentration of 3.1 μg/ml was active against *B. fragilis;* however, erythromycin was less active against the other groups. Most of the minimal inhibitory concentrations of lincomycin exceeded those of clindamycin by fourfold. Rifampin inhibited virtually all strains at 3.1 μg/ml.

The increasing recognition of the role of anaerobic bacteria in infections has made it necessary for the clinician to become familiar with the classification and nomenclature of this large group of organisms. In contrast to the clinically significant aerobic and facultatively anaerobic bacteria, the fastidious nature of the anaerobic bacteria precludes the prompt performance of antimicrobial susceptibility tests. Therefore, appropriate antimicrobial therapy is contingent on awareness of the possibility of infection by anaerobic bacteria and familiarity with susceptibility patterns of these bacteria.

This report presents the minimal inhibitory concentrations (MIC) of 601 clinical isolates of anaerobic bacteria to 10 different antimicrobial agents. In addition, the minimal bactericidal concentrations (MBC) of several antibiotics against 25 isolates of *Bacteroides fragilis* from blood will be presented.

MATERIALS AND METHODS

All anaerobic bacteria tested represented isolates from clinical material submitted to the Section of Clinical Microbiology during portions of an 8-month period beginning in November 1970 [for the total experience during this period, with classification by specimen source and species isolated, see report by Martin (16)]. Subcultures of anaerobic bacteria were made at the time of initial isolation and stored at −42 C according to the methods described by Dowell and Hawkins (2). The 601 strains examined in this study were selected primarily on the basis of source and frequency of isolation. In this laboratory, all specimens, other than those from the throat, sputum, vagina, stomach, urine, and stool, are examined routinely for the presence of anaerobes; anaerobic cultures are performed on other specimens only by special request.

Antimicrobial susceptibility testing was performed, with certain modifications, by the method of Finegold et al. (5), by using the agar-dilution technique and the inocula-replicator device of Steers et al. (18). Isolates were incubated anaerobically for 48 hr in thioglycolate medium (135-C, BBL) enriched with sterile rabbit serum and, when necessary, with menadione. The broth culture was diluted to provide an inoculum of 10^5 to 10^6 colony-forming units on the surface of the agar. Serial twofold dilutions of antibiotics were incorporated in brain-heart infusion (BHI) agar (BBL) with 5% sheep blood so as to yield final concentrations ranging from 0.1 to 25 μg/ml. These plates were prepared on the day before the test and stored overnight at room temperature, rather than at 4 C, to prevent the increased oxygen absorption which occurs at refrigerator temperatures. After inoculation, all plates were incubated, including controls with known MIC values, in an anaerobic incubator (National Appliance Co., Portland, Ore.) at 37 C using the GasPak (BBL) modification described by Gardner and Martin (8). The MIC, which was determined after 48 hr of incubation, was defined as the lowest concentration permitting no growth, a barely visible fine haze, or not more than one discrete colony (3).

The MBC of six antibiotics against 25 strains of *B.*

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fragilis isolated from blood were determined in the following manner. Twofold dilutions of penicillin G, tetracycline, chloramphenicol, lincomycin, clindamycin, and erythromycin were prepared in 1.0-ml amounts of BHI broth (BBL) containing 2% sheep blood. Forty-eight-hour broth cultures were diluted 1:100 in BHI broth containing 2% sheep blood; 1.0 ml of this suspension was added to each tube containing antibiotic so as to yield final concentrations ranging from 0.1 to 25 μg/ml. This inoculum provided adequate growth in the control tube (without antibiotic) within 48 hr. All tubes were incubated anaerobically (GasPak) at 37 C and examined at 48 hr. The MIC was defined as the lowest concentration of antibiotic that completely inhibited visible growth. MBC was determined by transferring 0.05 ml from each tube without visible or with barely visible growth to quarter sections of BHI agar plates (BBL) with 5% sheep blood and incubating anaerobically at 37 C for 48 hr. The lowest antibiotic concentration from which subcultures showed no growth represented the MBC.

Isolation, subculture, and most biochemical tests were carried out according to the procedures outlined by Dowell and Hawkins (2) using the GasPak (BBL) system. Prereduced media (Scott Laboratories, Inc., Chapel Hill, N.C.) were used for the differential carbohydrate fermentations. Identification and speciation of the anaerobic isolates was according to the criteria of Dowell and Hawkins (2).

RESULTS

With the exception of certain species of *Bacteroides*, penicillin G was highly active, against most anaerobes tested, at 6.2 μg/ml or less (Table 1). *Bacteroides incommunis*, *B. variabilis*, and *B. terebrans* were less susceptible; penicillin G exhibited little or no activity against *B. fragilis* at 6.2 μg/ml.

Cephalothin (Table 2) generally exhibited a similar degree of activity against the same species but at concentrations of 12.5 μg/ml or less. It is noteworthy that only 64% of strains of *Eubacterium lentum* were inhibited at this concentration, compared to 100% of the strains of *E. alactolyticum* and *Eubacterium* sp.

Tetracycline at 6.2 μg/ml was active against the majority of strains in most of the groups tested; some strains were resistant (Table 3). It is significant that only 39% of strains of *B. fragilis* and 20% of strains of *B. terebrans* were inhibited by this concentration. Although relatively few in number, 50% of the strains of *E. alactolyticum* and 20% of the *Eubacterium* sp. were inhibited by this concentration, whereas 85% of the strains of *E. lentum* were susceptible.

The data for chloramphenicol are shown in Table 4. At a concentration of 12.5 μg/ml, all but three of the strains tested were inhibited.

Erythromycin was active against many strains at a concentration of 3.1 μg/ml or less (Table 5). Less activity was exhibited against strains of *B. incommunis*, *B. variabilis*, *B. terebrans*, *E. alactolyticum*, *Eubacterium* sp., and *Veillonella*. Although both strains of *Catenabacterium* sp. were inhibited at 0.1 μg/ml, the three strains of

TABLE 1. *Susceptibility of anaerobic bacteria to penicillin G*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195				1		3	7	37	89
B. incommunis	10	10	40			50		60	70	
B. variabilis	5		40		60				80	100
B. oralis	2		50			100				
B. terebrans	5			20	40	80				
B. melaninogenicus	29	55	59	62	65	83		86	96	100
Bacteroides F1, F2, F3	11	36	54		73	82	91	100		
Fusobacterium fusiforme	18	61	89	94	100					
Fusobacterium sp.	2			50	100					
Clostridium perfringens	34	82	97				100			
Clostridium sp.	17	35	88	100						
Peptococcus sp.	145	48	91	95	96	97	99		100	
Peptostreptococcus sp.	72	58	91	97	98	100				
Veillonella sp.	13	23	77	100						
Propionibacterium acnes	16	94	100							
Eubacterium lentum	14	28	35	43	64	93	100			
E. alactolyticum	2	50				100				
Eubacterium sp.	5	40			60	100				
Bifidobacterium sp.	5		20	100						
Catenabacterium filamentosum	3		100							
Catenabacterium sp.	2	100								

TABLE 2. *Susceptibility of anaerobic bacteria to cephalothin*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195	1					2		4	9
B. incommunis	10	10				40				60
B. variabilis	5					40				
B. oralis	2	100								
B. terebrans	5						20			40
B. melaninogenicus	29	55	62	65	69			79	93	100
Bacteroides F1, F2, F3	11	18	27	45		63		82		100
Fusobacterium fusiforme	18	44	50	56		72		78		
Fusobacterium sp.	2				50				100	
Clostridium perfringens	34	8	17	70	96	100				
Clostridium sp.	17	23	35	88	100					
Peptococcus sp.	145	22	38	56	78	88	89	97	98	99
Peptostreptococcus sp.	72	40	57	70	78	96		99		100
Veillonella sp.	13	23	69		84	100				
Propionibacterium acnes	16	69	88		94	100				
Eubacterium lentum	14	7		28			43		64	85
E. alactolyticum	2	50						100		
Eubacterium sp.	5				20	40		60	100	
Bifidobacterium sp.	5				20		40		80	100
Catenabacterium filamentosum	3					33		100		
Catenabacterium sp.	2	50			100					

TABLE 3. *Susceptibility of anaerobic bacteria to tetracycline*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195	1	4	18	32	34	36	39	48	90
B. incommunis	10	10		30	70			80		90
B. variabilis	5			40	60					100
B. oralis	2			50				100		
B. terebrans	5						20			100
B. melaninogenicus	29	24	31	38	62	76	79	83	93	100
Bacteroides F1, F2, F3	11	18	27	45	54				63	91
Fusobacterium fusiforme	18	22	33	39	78	83		89		100
Fusobacterium sp.	2	50				100				
Clostridium perfringens	34	50	56	70		76	82	85	94	
Clostridium sp.	17	59	65	88			94	100		
Peptococcus sp.	145	12	15	29	52	56	59	62	74	89
Peptostreptococcus sp.	72	18	26	44	55	65	73	77	83	96
Veillonella sp.	13	23		31	69	76	85			100
Propionibacterium acnes	16	6		19	75	100				
Eubacterium lentum	14	7	21	57	64		71	85		100
E. alactolyticum	2							50	100	
Eubacterium sp.	5							20	40	60
Bifidobacterium sp.	5					40		60	80	100
Catenabacterium filamentosum	3				33	100				
Catenabacterium sp.	2				50		100			

TABLE 4. *Susceptibility of anaerobic bacteria to chloramphenicol*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195				1	2	23	98	100	
B. incommunis	10						60	100		
B. variabilis	5					20	80	100		
B. oralis	2					100				
B. terebrans	5				20	40	80			100
B. melaninogenicus	29	10	14		31	93	96	100		
Bacteroides F1, F2, F3	11			9	18	63	100			
Fusobacterium fusiforme	18	17		39	44	56	89	100		
Fusobacterium sp.	2			50		100				
Clostridium perfringens	34					15	100			
Clostridium sp.	17	6	12			53	88	100		
Peptococcus sp.	145	5	6	8	25	67	97	98		99
Peptostreptococcus sp.	72		3	11	37	63	96	100		
Veillonella sp.	13	15		23	46	85	100			
Propionibacterium acnes	16			12	31	94	100			
Eubacterium lentum	14	7		14		28	71	100		
E. alactolyticum	2					100				
Eubacterium sp.	5				20	60		100		
Bifidobacterium sp.	5					60	80	100		
Catenabacterium filamentosum	3						100			
Catenabacterium sp.	2		50		100					

TABLE 5. *Susceptibility of anaerobic bacteria to erythromycin*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195	13	16	26	39	66	92	95		97
B. incommunis	10	10	20		40	50	60			
B. variabilis	5					40				
B. oralis	2	100								
B. terebrans	5	60								
B. melaninogenicus	29	45	72	79	93	96				
Bacteroides F1, F2, F3	11	45	63	82		91				
Fusobacterium fusiforme	18	33	50	61	72	89				100
Fusobacterium sp.	2									
Clostridium perfringens	34			6	35	97	100			
Clostridium sp.	17	12		59	94	100				
Peptococcus sp.	145	9	10	16	24	58	79	80	86	87
Peptostreptococcus sp.	72	37	39	46	56	73	88	92	99	
Veillonella sp.	13		8	16		23	31	38	69	77
Propionibacterium acnes	16	88						94		
Eubacterium lentum	14	71	85						93	
E. alactolyticum	2	50								
Eubacterium sp.	5	20	60							
Bifidobacterium sp.	5	100								
Catenabacterium filamentosum	3									
Catenabacterium sp.	2	100								

TABLE 6. *Susceptibility of anaerobic bacteria to lincomycin*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195	12	13	16	31	71	82	95	99	
B. incommunis	10	10		20	50	60	70	90		
B. variabilis	5				40	80			100	
B. oralis	2	100								
B. terebrans	5				20	60	80			
B. melaninogenicus	29	89	96							
Bacteroides F1, F2, F3	11	63	73	91	100					
Fusobacterium fusiforme	18	39	50	56	67	83	94	100		
Fusobacterium sp.	2			50					100	
Clostridium perfringens	34	14	29	35	38	76	97	100		
Clostridium sp.	17	23	29		41	53	76	88	100	
Peptococcus sp.	145	29	45	69	92	95	96			97
Peptostreptococcus sp.	72	39	58	72	85	96	100			
Veillonella sp.	13	46	77			92		100		
Propionibacterium acnes	16	81	88	94		100				
Eubacterium lentum	14	21	28	43	71	93	100			
E. alactolyticum	2	100								
Eubacterium sp.	5	40	60				100			
Bifidobacterium sp.	5	40	80			100				
Catenabacterium filamentosum	3				66	100				
Catenabacterium sp.	2	100								

TABLE 7. *Susceptibility of anaerobic bacteria to clindamycin*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195	67	72	83	89	96	100			
B. incommunis	10	70			80	90		100		
B. variabilis	5	60				100				
B. oralis	2	100								
B. terebrans	5	60			80					
B. melaninogenicus	29	89	96			100				
Bacteroides F1, F2, F3	11	100								
Fusobacterium fusiforme	18	67	89	94		100				
Fusobacterium sp.	2	50								100
Clostridium perfringens	34	35	44	64	79	91	100			
Clostridium sp.	17	47	53	59	76	88	94		100	
Peptococcus sp.	145	62	76	87	94	95	96	97		
Peptostreptococcus sp.	72	81	85	90	98	100				
Veillonella sp.	13	100								
Propionibacterium acnes	16	88	100							
Eubacterium lentum	14	78	85		100					
E. alactolyticum	2	100								
Eubacterium sp.	5	60		100						
Bifidobacterium sp.	5	100								
Catenabacterium filamentosum	3	33	66	100						
Catenabacterium sp.	2	100								

TABLE 8. *Susceptibility of anaerobic bacteria to kanamycin*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195									
B. incommunis	10									10
B. variabilis	5									
B. oralis	2									
B. terebrans	5									20
B. melaninogenicus	29	24	27	34						41
Bacteroides F1, F2, F3	11		9			18				
Fusobacterium fusiforme	18	17							27	50
Fusobacterium sp.	2									50
Clostridium perfringens	34									6
Clostridium sp.	17	6								
Peptococcus sp.	145	1				2		4	13	44
Peptostreptococcus sp.	72				1	5	11	12	23	44
Veillonella sp.	13	8						23	38	61
Propionibacterium acnes	16					6			19	56
Eubacterium lentum	14				14	28	35		64	78
E. alactolyticum	2		50							
Eubacterium sp.	5	20								60
Bifidobacterium sp.	5									20
Catenabacterium filamentosum	3									
Catenabacterium sp.	2			50						

TABLE 9. *Susceptibility of anaerobic bacteria to gentamicin*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195								1	6
B. incommunis	10								10	40
B. variabilis	5									60
B. oralis	2							50	100	
B. terebrans	5			40					60	
B. melaninogenicus	29	24		31		34		45	59	76
Bacteroides F1, F2, F3	11	27	36						45	73
Fusobacterium fusiforme	18						5		11	56
Fusobacterium sp.	2									50
Clostridium perfringens	34									6
Clostridium sp.	17	6								18
Peptococcus sp.	145	2	3		5	7	11	30	62	95
Peptostreptococcus sp.	72	1	2	9	13	18	20	33	58	78
Veillonella sp.	13					8		31	46	85
Propionibacterium acnes	16						25	56	75	81
Eubacterium lentum	14		7	43	57	71		85	93	100
E. alactolyticum	2	50								
Eubacterium sp.	5	20						40		
Bifidobacterium sp.	5				20			60	80	100
Catenabacterium filamentosum	3									66
Catenabacterium sp.	2			50					100	

TABLE 10. *Susceptibility of anaerobic bacteria to rifampin*

Organism	Strains tested	Cumulative percentages at various concentrations (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	195	19	45	86	97	100				
B. incommunis	10	20	30	70	100					
B. variabilis	5	20	40	60	100					
B. oralis	2	50	100							
B. terebrans	5	60	80							
B. melaninogenicus	29	86	93	96						
Bacteroides F1, F2, F3	11	73	82	91		100				
Fusobacterium fusiforme	18	27	56	67	78	89	100			
Fusobacterium sp.	2									
Clostridium perfringens	34	97			100					
Clostridium sp.	17	82			88	100				
Peptococcus sp.	145	47	50	62	76	96	98	99		
Peptostreptococcus sp.	72	68			72	94		96		
Veillonella sp.	13	23			31	100				
Propionibacterium acnes	16	94				100				
Eubacterium lentum	14	78	85	93						
E. alactolyticum	2	50								
Eubacterium sp.	5	40				60				
Bifidobacterium sp.	5	60		80		100				
Catenabacterium filamentosum	3					100				
Catenabacterium sp.	2	100								

C. filamentosum were not inhibited by as much as 25 μg/ml.

In most instances, the results obtained with lincomycin resembled those obtained with erythromycin (Table 6). Clindamycin, on the other hand, exhibited a high degree of activity against all anaerobes at 1.6 μg/ml or less (Table 7). In general, the MIC of lincomycin exceeded those of clindamycin by fourfold.

Nearly all strains in this study were resistant to kanamycin and gentamicin, although both of these drugs showed some activity against the anaerobic cocci, the nonsporeforming gram-positive bacilli, and strains of *B. melaninogenicus* and *Fusobacterium fusiforme* (Tables 8 and 9).

With rifampin, virtually all strains were inhibited at 3.1 μg/ml or less, with the exception of some strains in the genus *Eubacterium* (Table 10).

The MBC of penicillin G (Table 11) and tetracycline (Table 12) against 25 strains of *B. fragilis* isolated from blood were generally four to eight times the MIC. The bactericidal concentration of chloramphenicol (Table 13) was eight or more times the bacteriostatic concentration, whereas with lincomycin (Table 14), clindamycin (Table 15), and erythromycin (Table 16), the MBC generally exceeded the MIC by at least 16-fold.

DISCUSSION

In contrast to the voluminous literature on in vitro susceptibility testing of clinically significant aerobic and facultatively anaerobic bacteria, only a few references exist with regard to the anaerobes, and most of these are concerned primarily with the nonsporeforming gram-negative bacilli (1, 9, 10, 14). In these studies involving anaerobic bacteria, several different techniques were used, thereby making comparisons difficult. Moreover, only a few antibiotics were studied. In some of these studies, species differences were not taken into account. Recently, in vitro studies by Finegold and colleagues (4–7, 17; Finegold et al., Bacteriol. Proc. 1965, p. 64; 1967, p. 96), Thornton and Cramer (19), and Ingham and associates (12, 13), among others, have developed meaningful data without the aforementioned shortcomings.

With few exceptions, the data reported here agree closely with those reported by these authors. Indeed, the MIC in this study for many of the nonsporeforming gram-negative bacilli, the *Peptostreptococcus* sp., and the *Bifidobacterium* sp. agreed well with those published by Finegold and associates (4, 6, 17; Finegold et al., Bacteriol. Proc. 1965, p. 64; 1967, p. 96). Moreover, the MIC of cephalothin against *Clostridium perfringens* showed excellent agreement with those recently reported by Traub (20). One particularly

TABLE 11. *Minimal bactericidal concentrations (MBC) against Bacteroides fragilis: penicillin G*

MIC (μg/ml)	MBC, μg/ml (no. of strains)[a]											
	>100	100	50	25	12.5	6.2	3.1	1.6	0.8	0.4	0.2	0.1
100	1											
50	4	2	2									
25	5			1								
12.5												
6.2												
3.1	1	1		1		1	2					
1.6			1					1				
0.8								1				
0.4												
0.2					1							
0.1												

[a] >100, Not bactericidal at 100 μg/ml or less; no concentrations greater than 100 μg/ml were tested.

TABLE 12. *Minimal bactericidal concentrations (MBC) against Bacteroides fragilis: tetracycline*

MIC (μg/ml)	MBC, μg/ml (no. of strains)[a]											
	>100	100	50	25	12.5	6.2	3.1	1.6	0.8	0.4	0.2	0.1
100												
50	1		2									
25	3		2	2								
12.5	3		1	1								
6.2	2					1						
3.1												
1.6							1					
0.8												
0.4												
0.2	1											
0.1	2	1	1									1

[a] >100, Not bactericidal at 100 μg/ml or less; no concentrations greater than 100 μg/ml were tested.

TABLE 13. *Minimal bactericidal concentrations (MBC) against Bacteroides fragilis: chloramphenicol*

MIC (μg/ml)	MBC, μg/ml (no. of strains)[a]											
	>100	100	50	25	12.5	6.2	3.1	1.6	0.8	0.4	0.2	0.1
100	1											
50												
25												
12.5	5											
6.2	7			1	1							
3.1	3	1										
1.6	1		1				2					
0.8	1											
0.4												
0.2			1									
0.1												

[a] >100, Not bactericidal at 100 μg/ml or less; no concentrations greater than 100 μg/ml were tested.

TABLE 14. *Minimal bactericidal concentrations (MBC) against Bacteroides fragilis: lincomycin*

MIC (μg/ml)	MBC, μg/ml (no. of strains)[a]											
	>100	100	50	25	12.5	6.2	3.1	1.6	0.8	0.4	0.2	0.1
100												
50												
25												
12.5												
6.2			1									
3.1		1	1	2			4					
1.6	4				1							
0.8	2								1			
0.4	4			1								
0.2									1			
0.1						1					1	

[a] >100, Not bactericidal at 100 μg/ml or less; no concentrations greater than 100 μg/ml were tested.

TABLE 15. *Minimal bactericidal concentrations (MBC) against Bacteroides fragilis: clindamycin*

MIC (μg/ml)	MBC, μg/ml (no. of strains)[a]											
	>100	100	50	25	12.5	6.2	3.1	1.6	0.8	0.4	0.2	0.1
100												
50												
25												
12.5												
6.2												
3.1						1						
1.6					1							
0.8						1		1				
0.4									1			
0.2											1	
0.1	6	1	1	3	1		1	1		1	1	3

[a] >100, Not bactericidal at 100 μg/ml or less; no concentrations greater than 100 μg/ml were tested.

TABLE 16. *Minimal bactericidal concentrations (MBC) against Bacteroides fragilis: erythromycin*

MIC (μg/ml)	MBC, μg/ml (no. of strains)[a]											
	>100	100	50	25	12.5	6.2	3.1	1.6	0.8	0.4	0.2	0.1
100	1											
50												
25												
12.5												
6.2	1					1						
3.1	1											
1.6	3						1	1				
0.8	5		1	1								
0.4	4					1						
0.2	2			1					1			
0.1												

[a] >100, Not bactericidal at 100 μg/ml or less; no concentrations greater than 100 μg/ml were tested.

noteworthy area of disagreement in this study is the activity of tetracycline against *B. fragilis*. Several of the aforementioned investigators reported that *B. fragilis* was sensitive to tetracycline. For example, with the agar-dilution technique, Ingham et al. (12) found that all 17 strains tested were inhibited at 0.82 μg/ml or less, whereas Finegold and Hewitt (7) found that 90% of their strains of *B. fragilis* were sensitive to tetracycline at concentrations of 1.56 μg/ml or less. In our study, this drug inhibited only 39% of strains of *B. fragilis* at 6.2 μg/ml or less. Some of the differences can be attributed to differences in procedure; however, it is conceivable that *B. fragilis* is becoming more resistant to tetracycline. Recent in vitro studies by Finegold and associates (*personal communication*), as well as by others (1, 14, 19), suggest that many of their recent isolates are noted to be resistant to this drug.

The MIC of penicillin G against the 29 strains of *B. melaninogenicus* in this study are of interest in that 83% of these were inhibited at concentrations of 3.1 μg/ml or less. Finegold et al. (6) reported in 1967 that all 19 of their strains were completely inhibited at concentrations of 0.8 μg/ml or less. It is difficult to reconcile this fourfold difference, since the data on the MIC of lincomycin and erythromycin against this species appear to be in close agreement. Our data showing the antibacterial effect of both clindamycin and rifampin against strains of *B. fragilis* are in good agreement with the MIC reported by Ingham et al. (13).

The effect of CO_2 on the susceptibility of 10 strains of *B. fragilis* to four antibiotics in vitro was recently reported by Ingham et al. (13). They found that the MIC of erythromycin and lincomycin were 4 to 32 times higher when grown in hydrogen plus 10% CO_2 than when grown in pure hydrogen. Clindamycin and rifampin, to which their strains of *B. fragilis* were uniformly sensitive, were not affected by additional CO_2. Although none of our strains were incubated in pure hydrogen, the activities of erythromycin and lincomycin against our strains of *B. fragilis* were in general agreement with those in antimicrobial susceptibility tests performed with incubation in an environment containing 5 to 10% CO_2 (6, 9–11). This observation is not surprising since the GasPak (BBL) system used in this study for anaerobic incubation (8) provides an atmosphere containing 8 to 10% CO_2 (the remainder being hydrogen gas) once the generator envelope is activated by the addition of water (D. A. Power, BBL, *personal communication*). The efficacy of both lincomycin and erythromycin in the treatment of infections caused by *Bacteroides* sp., despite the CO_2 present in the body, has been reported (13).

More than half of the 25 strains of *B. fragilis* tested failed to be killed at concentrations of each of six antibiotics attainable in serum at their normally recommended dosages. Bactericidal activity of these antibiotics tended to be inconsistent. These results are in disagreement with those obtained by Ingham et al. (12) using a replica-plating technique with velvet pads; however, it is likely that Ingham et al. used a smaller inoculum of bacteria in the inhibitory phase of their test than we did. Moreover, their definition of significant growth was 20 or more colonies in subcultures of plates with no growth or with growth of not more than 19 colonies. Our MBC was defined by the absence of any growth on subculture of broth containing no or barely visible growth. These differences in results emphasize the desirability of standardization of the techniques and interpretations of bactericidal tests.

Although tetracycline has been considered to be the agent of choice in the treatment of infections due to penicillin-resistant strains of *Bacteroides* (4, 11, 13), our data and those of others (1, 14, 19) demonstrating substantial resistance of these organisms to this antibiotic and its inconsistent bactericidal activity raise serious questions about this recommendation. The efficacy of chloramphenicol in the treatment of *Bacteroides* sepsis, however, has also been questioned recently by Kagnoff and Armstrong (15). The need for a well-controlled prospective clinical study of antibiotic efficacy in bacteremia due to anaerobic bacteria is clear.

The antimicrobial susceptibility data accumulated from these 601 clinical isolates of anaerobic bacteria indicate certain definite patterns that should be helpful in the selection of appropriate antibacterial therapy. With the possible exception of blood culture isolates, we do not think at this time that routine antibiotic susceptibility testing can be performed with the same facility and frequency as can be performed on the aerobic bacteria. Periodic testing, however, probably should be carried out to detect any significant changes in patterns of resistance that may develop. Data correlating results of disc-diffusion susceptibility testing with MIC would be helpful in simplifying the routine susceptibility testing of anaerobic bacteria.

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