ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1976, p. 736-752 Vol. 10, No. 4

Susceptibility of Anaerobic Bacteria to 23 Antimicrobial Agents

VERA L. SUTTER* AND SYDNEY M. FINEGOLD

Research and Medical Services, Veterans Administration Wadsworth Hospital Center, Los Angeles, California 90073, and Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024*

Received for publication 23 June 1976

The antimicrobial susceptibility of 492 anaerobic bacteria, the majority of which were recent clinical isolates, was determined by the agar dilution technique. Penicillin G was active against most of the strains tested at 32 U or less/ml, but only 72% of *Bacteroides fragilis* strains were susceptible at this level and 9% required 256 U or more/ml. Ampicillin was effective against most of the strains except *B. fragilis* at 16 μg or less/ml. Amoxicillin was active against only 31% of *B. fragilis*, 76% of other *Bacteroides* species, and 67% of *Fusobacterium* species at 8 μg/ml. Two new penicillins, mezlocillin and azlocillin, were similar to ampicillin in their activity. Carbenicillin and ticarcillin inhibited all but a few strains at 128 μg or less/ml. BLP 1654 was somewhat more active than penicillin G against *B. fragilis* but had similar activity against other anaerobes. Cephalothin was inactive against *B. fragilis*, and only 65% of other *Bacteroides* species were inhibited by 32 μg or less/ml. It was effective against all other anaerobes at that level. Cefamandole showed somewhat greater activity than cephalothin against *B. fragilis* but generally less activity against gram-positive organisms. Cefazaflur (SKF 59962) was comparable to cephalothin against *B. fragilis*. Cefoxitin was distinctly more active than cephalothin against *B. fragilis*. These latter two agents were less active than cephalothin against the gram-positive anaerobes. Chloramphenicol remains active against anaerobic bacteria at 16 μg or less/ml, with rare exceptions. Thiamphenicol was similar to chloramphenicol in its activity. Clindamycin was very active against most of the anaerobes at 8 μg or less/ml. Erythromycin and josamycin were also tested, with josamycin showing greater activity against *B. fragilis* than either erythromycin or clindamycin. A new oligosaccharide, everninomicin B, was less active than clindamycin against *B. fragilis* but more active against clostridia and some of the other strains tested. Most of the groups of bacteria tested demonstrated a trend toward resistance to tetracycline. Doxycyline and minocycline were somewhat more active than was tetracycline. Metronidazole was active against the majority of the anaerobes tested; resistance ws demonstrated by some of the gram-positive cocci and gram-positive, non-sporeforming bacilli.

It has been generally accepted by investigators and clinicians knowledgeable in the diagnosis and therapy of anaerobic infections that rapid, routine susceptibility testing of individual isolates of anaerobic bacteria is impractical. Anaerobic infections are frequently polymicrobic and cultures require relatively long periods of time for growth and isolation, so that timely results of susceptibility tests are not feasible. It has been suggested that centers with the capability for testing on a survey basis do so periodically to monitor changing patterns of resistance to commonly recommended agents and to determine the possible effectiveness of newer agents.

The purpose of the present study was to assay the in vitro effect of several drugs currently in use for therapy of anaerobic infections and to determine the activity of several newer agents against anaerobic bacteria recently isolated from clinical material.

MATERIALS AND METHODS

Bacterial strains. A total of 492 strains of anaerobic bacteria was used throughout the study. All but a few were isolated from a variety of clinical specimens received by the Wadsworth Anaerobic Bacteriology Research Laboratory from patients on special studies and from patients seen in consultation by the Infectious Disease Section during the period of September 1972 to June 1975. Exceptions were 21 strains of *Bacteroides melaninogenicus*, 5 of which were reference strains and 16 of which were normal oral or fecal isolates. Table 1 gives the specific iden-

TABLE 1. *Identity of bacterial strains tested*

Organism	No. of strains tested in studies		
	Part I	Part II	Total
Bacteroides fragilis[a]	42	34	76
subsp. *distasonis*	8	3	11
subsp. *fragilis*	18	12	30
subsp. *ovatus*	1	2	3
subsp. *thetaiotaomicron*	9	12	21
subsp. *vulgatus*	4	4	8
Other	2	1	3
Bacteroides melaninogenicus	60	11	71
subsp. *asaccharolyticus*	21	1	22
subsp. *intermedius*	29	6	35
subsp. *melaninogenicus*	10	2	12
Other	0	2	2
Other *Bacteroides* and *Selenomonas*	21	51	72
B. capillosus	0	3	3
B. clostridiiformis subsp. *clostridiiformis*[b]	0	2	2
B. clostridiiformis subsp. *girans*[b]	0	1	1
B. corrodens	2	0	2
B. oralis	4	6	10
B. pneumosintes	2	3	5
B. putredinis	1	1	2
B. ruminicola subsp. *ruminicola*	0	1	1
B. ruminicola subsp. *brevis*	1	3	4
B. splanchnicus[c]	1	0	1
Bacteroides group I	0	6	6
Bacteroides group PS	0	8	8
Bacteroides sp.	10	16	26
Selenomonas sp.	0	1	1
Fusobacterium nucleatum	8	10	18
Other *Fusobacterium*	12	4	16
F. gonidiaformans	2	2	4
F. mortiferum	2	0	2
F. naviforme	1	1	2
F. necrophorum	1	0	1
F. russii	1	0	1
Fusobacterium sp.	5	1	6
Peptococcus and *Gaffkya*	17	42	59
P. asaccharolyticus	2	21	23
P. magnus	4	6	10
P. prevotii	6	8	14
P. saccharolyticus	0	2	2
P. variabilis	4	4	8
Peptococcus sp.	1	0	1
Gaffkya anaerobius	0	1	1
Peptostreptococcus	15	14	29
P. anaerobius	9	9	18
P. micros	5	4	9
Peptostreptococcus sp.	1	1	2
Microaerophilic and anaerobic *Streptococcus*	6	4	10
S. constellatus	0	2	2
S. intermedius	4	2	6
S. morbillorum	2	0	2
Gram-negative cocci	7	19	26
Acidaminococcus fermentans	2	1	3
Veillonella alcalescens	2	6	8
V. parvula	3	12	15
Eubacterium	8	9	17
E. aerofaciens	0	1	1
E. contortum	0	1	1
E. lentum	4	4	8
E. limosum	2	0	2
E. moniliforme	1	0	1
Eubacterium sp.	1	3	4
Arachnia propionica	2	1	3
Propionibacterium	4	8	12
P. acnes	4	5	9
P. avidum	0	2	2
Propionibacterium sp.	0	1	1
Actinomyces	16	0	16
A. israelii	1	0	1
A. naeslundii	5	0	5
A. viscosus	1	0	1
Actinomyces sp.	9	0	9
Bifidobacterium	0	5	5
B. adolescentis var. B	0	2	2
B. infantis	0	1	1
Bifidobacterium sp.	0	2	2
Lactobacillus	10	9	19
L. acidophilus	0	2	2
L. catenaforme	2	1	3
L. fermentum	0	1	1
L. minutis	1	2	3
L. plantarum	2	1	3
Lactobacillus sp.	5	2	7
Clostridium perfringens	8	1	9
Other *Clostridium*	27	7	34
C. bifermentans	2	0	2
C. butyricum	1	0	1
C. difficile	2	0	2
C. glycolicum	1	0	1
C. innocuum	5	3	8
C. paraperfringens	1	0	1
C. plagarum	1	0	1
C. putrificum	1	0	1
C. ramosum	7	3	10
C. sphenoides	1	0	1
C sporogenes	0	1	1
C. tertium	1	0	1
Clostridium sp.	4	0	4

[a] It has recently been proposed that organisms recognized as subspecies of *B. fragilis* be reinstated to species rank (7).

[b] Strains having phenotypic characteristics similar to these have recently been found to have spores and to be genetically similar to *Clostridium*. It has been proposed that they be considered as *C. clostridiiformis* (15) or *C. clostridiiforme* (8). Spores have not been demonstrated in the strains in the present study.

[c] A new *Bacteroides* species described by Werner et al. (48).

tification of the bacteria used in each part of the study. These bacteria were identified according to the criteria of the *Wadsworth Anaerobic Bacteriology Manual* (40) and the Virginia Polytechnic Institute *Anaerobe Laboratory Manual* (13). It has recently been proposed that organisms recognized as subspecies of *Bacteroides fragilis* be given species status (7). We are in agreement with this, but since little or no difference has been found in the susceptibility of these organisms to the antimicrobial agents in the present study and in reports of others (2, 14), we will refer to *B. fragilis* as a group including the five species. The subspecies of *B. melaninogenicus* are also referred to as a group, because no differences were observed in their susceptibility to antimicrobial agents. Six strains had the characteristics of *Bacteroides* group 1 and eight strains had the characteristics of *Bacteroides* group PS. These latter two groups have recently been recognized as distinct from other *Bacteroides* species (L. V. Holdeman, personal communication).

Antimicrobial agents. Laboratory-standard powders were kindly supplied as follows: penicillin G, cephalothin, cefamandole lithium, and erythromycin from Eli Lilly & Co., Indianapolis, Ind.; ampicillin trihydrate and BLP 1654 from Bristol Laboratories, Syracuse, N.Y.; carbenicillin from Roerig, New York, N.Y.; amoxicillin trihydrate and ticarcillin sodium from Beecham-Massengill Pharmaceuticals, Bristol, Tenn.; mezlocillin and azlocillin from Delbay Pharmaceuticals, Inc., Bloomfield, N.J.; sodium cefoxitin from Merck, Sharp and Dohme, Rahway, N.J.; cefazaflur (SKF 59962) from Smith, Kline and French Laboratories, Philadelphia, Pa.; chloramphenicol from Parke-Davis & Co., Detroit, Mich.; thiamphenicol and thiamphenicol glycinate from Zambon, S.p.A., Bresso, Milano, Italy; clindamycin from The Upjohn Co., Kalamazoo, Mich.; everninomicin B from Schering Corp., Bloomfield, N.J.; josamycin from E. I. Dupont de Nemours & Co., Newark, Del.; tetracycline and doxycycline from Pfizer Laboratories, New York, N.Y.; minocycline from Lederle Laboratories, Pearl River, N.Y.; and metronidazole from G. D. Searle & Co., Chicago, Ill.

Procedures. Antimicrobial susceptibility testing was performed by the agar dilution technique as previously described (40). Sets of three laked blood agar plates containing no antibiotic were inoculated before each series of antibiotic-containing plates and incubated anaerobically, in 10% CO_2 in air, and aerobically, to serve as growth and contamination controls. Data were deleted if poor or no growth occurred on the anaerobic growth control plate or if contamination was evident.

Throughout the study, the following antimicrobial agents were included: penicillin G, carbenicillin, BLP 1654, chloramphenicol, clindamycin, tetracycline, doxycycline, minocycline, and metronidazole. During the first part of the study, amoxicillin, ticarcillin, cefoxitin, cefazaflur, thiamphenicol, thiamphenicol glycinate, and everninomicin B were included to determine their possible effectiveness against anaerobes or for comparison of their activity with other drugs. During the second part of the study, the following agents were included: ampicillin, mezlocillin, azlocillin, cephalothin, cefamandole, erythromycin, and josamycin.

RESULTS AND DISCUSSION

Penicillins. The activity of penicillin G against the anaerobes tested is shown in Table 2. An inhibitory concentration of 32 U/ml is considered an acceptable level of susceptibility

TABLE 2. *Activity of penicillin G against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (U/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	76	1	3		5	7	13	42	72	91		100
Bacteroides melaninogenicus	66	68	79	83	86	89	94	97	100			
Other *Bacteroides* and *Selenomonas*	72	24	49	51	56	63	71	75	82	93	96	100
Fusobacterium nucleatum	18	72	83	94		100						
Other *Fusobacterium*	16	50	75		88						94	100
Peptococcus and *Gaffkya*	59	49	100									
Peptostreptococcus	29	55	86	90			97		100			
Anaerobic and microaerophilic streptococci	10	50	90					100				
Gram-negative cocci	26	15	69	85	89	92	100					
Eubacterium	17	18	47	59	77	94	100					
Arachnia propionica	3		67	100								
Propionibacterium	12	75	92		100							
Actinomyces	16	69	100									
Bifidobacterium	5	20	100									
Lactobacillus	18	28	89		100							
Clostridium perfringens	9	33	100									
Other *Clostridium*	34	3	53	71	82	97	100					

[a] Includes all strains identified as subspecies of *B. fragilis*.

if a high parenteral dosage is given. Most of the bacteria were inhibited by this concentration or less. However, only 72% of *B. fragilis* strains, 82% of *Bacteroides* species other than *B. fragilis* and *B. melaninogenicus*, and 88% of *Fusobacterium* species other than *Fusobacterium nucleatum* were inhibited by 32 U/ml. Nine percent, or seven of the strains in the *B. fragilis* group (six *B. fragilis* and one *Bacteroides vulgatus*), 4% or three strains of other *Bacteroides* species, and one *Fusobacterium* species required 256 U or more/ml for inhibition. The *B. fragilis* strains included in this study appear to have about the same susceptibility as those tested in a prior study in which strains had been isolated from a variety of sources, including human feces, over a period of several years (39). There was no difference in susceptibility of strains tested in the two parts of the study, with approximately equal proportions of the resistant strains appearing in each segment. The present results with *B. fragilis* and other anaerobes tested are also similar to those reported by others (2, 14, 16, 21, 34, 43, 51), and greater resistance to penicillin does not appear to be emerging.

The activity of ampicillin against the strains tested in the second part of the study is shown in Table 3. With parenteral dosage, levels of at least 16 μg/ml are achievable. Ampicillin inhibited most of the anaerobes tested at concentrations of 16 μg or less/ml but was effective against only 56% of *B. fragilis* strains at this level. The results with *B. fragilis* are similar to those reported by Kislak (16) and overall results show higher minimum inhibitory concentrations (MICs) than those reported by Rotilie et al. (32), who used a microdilution technique for determination of susceptibility.

Amoxicillin, an oral, broad-spectrum penicillin, achieves serum levels of up to 8 μg/ml (24). The activity of this antibiotic against anaerobes was determined during the first part of the study. Amoxicillin was active against many of the strains tested (Table 4), but only 31% of *B. fragilis*, 76% of other *Bacteroides* species, and 67% of *Fusobacterium* species were inhibited by 8 μg or less/ml. One strain each of *Peptostreptococcus anaerobius* and *Streptococcus intermedius* required 16 μg/ml and one strain of *Clostridium perfringens* required 32 μg/ml for inhibition. These studies would indicate that amoxicillin would be less useful in serious anaerobic infections than either penicillin G or ampicillin.

In the second part of the study two new semisynthetic acylureido penicillins, mezlocillin and azlocillin, were tested. Results are shown in Tables 5 and 6. These compounds have similar in vitro effectiveness against the anaerobes tested and, on the basis of weight, are similar to ampicillin in their activity. Pharmacological data on these two compounds are not yet available.

The susceptibility of anaerobes to carbenicillin was tested throughout the study, and results are shown in Table 7. Carbenicillin inhibited all anaerobes tested at a level of 128 μg/

TABLE 3. *Activity of ampicillin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	34					6	15	56	82	91		100
Bacteroides melaninogenicus	9	22	67		78	100						
Other *Bacteroides* and *Selenomonas*	50	26	44	48	54	60	72	92	94	98		100
Fusobacterium nucleatum	10	70	80					90	100			
Other *Fusobacterium*	4	25	50				75					100
Peptococcus and *Gaffkya*	41	39	93	100								
Peptostreptococcus	13	23	85				92	100				
Anaerobic and microaerophilic streptococci	4	25	100									
Gram-negative cocci	19	16	79		90	95						100
Eubacterium	9	22	67	78	100							
Arachnia propionica	1			100								
Propionibacterium	7	29	100									
Bifidobacterium	5		100									
Lactobacillus	8	13	75	88					100			
Clostridium perfringens	1	100										
Other *Clostridium*	7	14	71	86	100							

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 4. *Activity of amoxicillin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	42	2	5	10	17	26	31	52	83	91	93	100
Bacteroides melaninogenicus	59	71	83	86	90	95	100					
Other *Bacteroides* and *Selenomonas*	21	43	57	62	67	76		86		95	100	
Fusobacterium nucleatum	8	63	88		100							
Other *Fusobacterium*	12	42	67					75		83	92	100
Peptococcus and *Gaffkya*	19	42	90	95	100							
Peptostreptococcus	15	53	87	93				100				
Anaerobic and microaerophilic streptococci	6	67	83					100				
Gram-negative cocci	7	57	86				100					
Eubacterium	7	57	100									
Arachnia propionica	2		100									
Propionibacterium	4	25	75	100								
Actinomyces	16	63	100									
Lactobacillus	10	60	90		100							
Clostridium perfringens	8	75	88						100			
Other *Clostridium*	26	8	77	92	96		100					

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 5. *Activity of mezlocillin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)											
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	256.0	≥512
Bacteroides fragilis[a]	34						6	27	62	82	91		100
Bacteroides melaninogenicus	9		67		78	89		100					
Other *Bacteroides* and *Selenomonas*	50	10	28	40	50	62	80	88	94	98			100
Fusobacterium nucleatum	10	60	90								100		
Other *Fusobacterium*	4	25	50	75			100						
Peptococcus and *Gaffkya*	42	48	95		100								
Peptostreptococcus	13		70		77	85		100					
Anaerobic and microaerophilic streptococci	4	50	75	100									
Gram-negative cocci	19			11	26	47	68	74	84			90	100
Eubacterium	9		22	44	78				100				
Arachnia propionica	1					100							
Propionibacterium	7	29	86	100									
Bifidobacterium	5	20	80	100									
Lactobacillus	8	13	63	88			100						
Clostridium perfringens	1		100										
Other *Clostridium*	7		29	43	86	100							

[a] Includes all strains identified as subspecies of *B. fragilis*.

ml, with the exception of four strains of *B. fragilis*, a *Bacteroides* species, a *Bacteroides corrodens*, a *Bacteroides clostridiiformis* subsp. *clostridiiformis*, and a *Fusobacterium* species. The MICs of strains in the present study are similar to those of our previous studies (36) and to those of Kislak (16), Staneck and Washington (34), and Zabransky and co-workers (51) but are appreciably lower than those of Blazevic and Matsen (3) and Tally et al. (43). Both of the latter studies utilized prereduced media containing cysteine in determining susceptibility; this might account for some loss of carbenicillin activity.

The activity of ticarcillin against the strains tested in the second part of the study is shown

TABLE 6. *Activity of azlocillin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)											
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	256.0	≥512
Bacteroides fragilis[a]	34						6	32	65	91		97	100
Bacteroides melaninogenicus	9	11	56		67	89	100						
Other *Bacteroides* and *Selenomonas*	50	12	38	42	50	54	70	80	88	94	98		100
Fusobacterium nucleatum	10	60	90							100			
Other *Fusobacterium*	4	25	50		75	100							
Peptococcus and *Gaffkya*	42	33	95	100									
Peptostreptococcus	13		69	77	85				100				
Anaerobic and microaerophilic streptococci	4	25	100										
Gram-negative cocci	19			5	37	63	79	84			95		100
Eubacterium	9	11	33	44	78				100				
Arachnia propionica	1					100							
Propionibacterium	7	29	100										
Bifidobacterium	5		80	100									
Lactobacillus	8		63	88		100							
Clostridium perfringens	1	100											
Other *Clostridium*	7		57	86	100								

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 7. *Activity of carbenicillin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)											
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	256.0	≥512
Bacteroides fragilis[a]	76	1	3	5	8	21	25	38	47	80	95		100
Bacteroides melaninogenicus	67	42	85	90	94	97	100						
Other *Bacteroides* and *Selenomonas*	72	7	47	53	68	76	81	88	92	96			100
Fusobacterium nucleatum	18	39	83			94	100						
Other *Fusobacterium*	16	25	44	50	81	88		94					100
Peptococcus and *Gaffkya*	59	25	73	88	98	100							
Peptostreptococcus	29	10	45	69	83	90		93		97	100		
Anaerobic and microaerophilic streptococci	10	20	40	50	80	90					100		
Gram-negative cocci	26		23	42	65	81	89	92	96		100		
Eubacterium	17	12	18	24	47	53	65	88	100				
Arachnia propionica	3				67		100						
Propionibacterium	12	17	83	92					100				
Actinomyces	16	69	100										
Bifidobacterium	5	20		40	60	100							
Lactobacillus	18	17	33	50	89	94		100					
Clostridium perfringens	9		100										
Other *Clostridium*	33		9	21	36	49	61	76	94	100			

[a] Includes all strains identified as subspecies of *B. fragilis*.

in Table 8. With high parenteral dosage schedules, blood levels of ticarcillin are similar to those of carbenicillin (26). The in vitro activity against anaerobes is also quite similar. It was effective against all strains tested at 128 μg or less/ml, with the exception of two strains of *B. fragilis* and one strain each of *Fusobacterium* species and *Fusobacterium gonidiaformans*.

Table 9 indicates the activity of BLP 1654 against the anaerobic bacteria tested throughout the study. Organisms with MICs of 32 μg or less/ml are considered susceptible to this agent (4). Unfortunately, because of its nephrotoxicity, further evaluation of BLP 1654 has been discontinued.

Cephalosporins and similar drugs. The susceptibility of the anaerobic bacteria from the second part of the study to cephalothin is shown in Table 10. All strains of *B. fragilis* were resistant to cephalothin, requiring at least 64 μg/ml for inhibition. Only 65% of other *Bacteroides* species were inhibited by 32 μg/ml. Previous studies indicated 6 to 11% of *B. fragilis* and all strains of other *Bacteroides* species to be inhibited by this concentration (36, 42). All other groups tested in the present study were inhibited by 32 μg or less/ml. Most of the gram-positive anaerobes were inhibited by 8 μg or

TABLE 8. *Activity of ticarcillin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	42	2		5	12	14	24	33	50	76	95	100
Bacteroides melaninogenicus	59	41	85	87	90	95	98	100				
Other *Bacteroides* and *Selenomonas*	21	24	48	62	67	71		86	95		100	
Fusobacterium nucleatum	8	38	88			100						
Other *Fusobacterium*	12	25	42	50	58	67	75	83				100
Peptococcus and *Gaffkya*	15	20	60	93	100							
Peptostreptococcus	15	13	53	80	93						100	
Anaerobic and microaerophilic streptococci	6		50			83			100			
Gram-negative cocci	7			29	57	71	86		100			
Eubacterium	7		14	29	43		57	86	100			
Arachnia propionica	2			50	100							
Propionibacterium	4		75						100			
Actinomyces	16	13	81	100								
Lactobacillus	10	10	60	70		80	90	100				
Clostridium perfringens	8		88	100								
Other *Clostridium*	26		8	15	31	54		85	96	100		

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 9. *Activity of BLP 1654 against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	76	1	3	4	5	8	32	63	90	91	93	100
Bacteroides melaninogenicus	68	50	81	85	87	93	99	100				
Other *Bacteroides* and *Selenomonas*	70	20	46	51	53	56	64	79	93	94	96	100
Fusobacterium nucleatum	18	72	78		83		89		100			
Other *Fusobacterium*	16	31	56			81				88		100
Peptococcus and *Gaffkya*	59	39	95	100								
Peptostreptococcus	28	54	89		93		96	100				
Anaerobic and microaerophilic streptococci	10	30	70	90				100				
Gram-negative cocci	26	8	39	58	73	89			92	96		100
Eubacterium	16	19	50	63	75		100					
Arachnia propionica	3		67		100							
Propionibacterium	11	46	91				100					
Actinomyces	16	25	100									
Bifidobacterium	5		100									
Lactobacillus	18	39	83	94					100			
Clostridium perfringens	9	22	100									
Other *Clostridium*	33		33	70	82	94	100					

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 10. *Activity of cephalothin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)											
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	256.0	≥512
Bacteroides fragilis[a]	34									15	50	53	100
Bacteroides melaninogenicus	9	44	56			67		78	100				
Other *Bacteroides* and *Selenomonas*	51	2	24	39	41	43	45	51	65	78	92	100	
Fusobacterium nucleatum	10	60	90	100									
Other *Fusobacterium*	4	50	100										
Peptococcus and *Gaffkya*	42	14	74	95	98		100						
Peptostreptococcus	14	14	79		93		100						
Anaerobic and microaerophilic streptococci	4	25	100										
Gram-negative cocci	19		68	84	90	100							
Eubacterium	9	11	33	44	67				100				
Arachnia propionica	1				100								
Propionibacterium	8		88	100									
Bifidobacterium	5		20		40	60	100						
Lactobacillus	8	13	100										
Clostridium perfringens	1			100									
Other *Clostridium*	7		14			29	100						

[a] Includes all strains identified as subspecies of *B. fragilis*.

less/ml. These data are in agreement with those of our earlier study, except that resistant clostridia have not been found as yet.

The activity of cefamandole (7-D-mandelamido-3-[1-methyl-1H-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid) was compared with that of cephalothin. Cefamandole is a new cephalosporin which has been shown to have a broad spectrum of activity against facultative bacteria (5, 22, 25). It has been shown to be resistant to penicillinase and cephalosporinases derived from facultative bacteria (25, 49). The results of the studies on the activity of cefamandole are shown in Table 11. Since cefamandole is resistant to β-lactamases, the strains of *B. fragilis* tested were more resistant to this agent than would be expected, with only 9% of the strains susceptible to 32 μg/ml. *B. fragilis* β-lactamases have been shown to be different from those produced by *Enterobacteriaceae* (29). It is possible that cefamandole is not resistant to the cephalosporinase produced by these strains. Our results with *B. fragilis* are in disagreement with those recently published by Ernst et al. (12), who found that 57% of their strains were inhibited by 32 μg of cefamandole per ml. This discrepancy could be due to an inoculum effect, since our inoculum is at least 10-fold greater than that used by Ernst and coworkers, or it could be due to carryover of a larger amount of cephalosporinase with the inoculum. The studies of Olsson and co-workers (29) showed that the amount of β-lactamase produced by their strains of *B. fragilis* was medium dependent, and it is possible that greater amounts of the enzyme are produced in the thioglycolate medium used in our studies. Further investigation is necessary to determine the significance of this phenomenon. Cefamandole was more active against *B. melaninogenicus* and other *Bacteroides* species than was cephalothin, had similar activity against *Fusobacterium* species, and was less active against the gram-positive anaerobes. Prior studies with cefazolin indicated that it may be slightly more active than cefamandole against *B. fragilis*, the anaerobic cocci, and clostridia (36).

The activities of cefazaflur (7-trifluoromethylthioacetamido-3-[1-methyl-1H-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid) and sodium cefoxitin were compared. Cefoxitin is a cephamycin, a class of compounds closely related to the cephalosporins. Results are shown in Tables 12 and 13. Cefazaflur was less active than cefoxitin against *B. fragilis* and other gram-negative anaerobes, was similar in activity against gram-positive, non-sporeforming anaerobes, and was somewhat more active against *Clostridium* species other than *C. perfringens*. Pharmacological studies with animals indicate that serum levels with cefazaflur are similar to those obtained with cephalothin

TABLE 11. *Activity of cefamandole against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)											
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	256.0	≥512
Bacteroides fragilis[a]	34								9	29	56	71	100
Bacteroides melaninogenicus	9	11	56	67		89	100						
Other *Bacteroides* and *Selenomonas*	51	8	24	35	51	55	67	76	90	94	98	100	
Fusobacterium nucleatum	10	80	100										
Other *Fusobacterium*	4	75	100										
Peptococcus and *Gaffkya*	42	17	57	83	88	93	98			100			
Peptostreptococcus	14	7	71	79			86	93	100				
Anaerobic and microaerophilic streptococci	4	25	100										
Gram-negative cocci	19	11		84	95			100					
Eubacterium	9	11	56	78				89	100				
Arachnia propionica	1				100								
Propionibacterium	8	13	100										
Bifidobacterium	5	20	60		100								
Lactobacillus	8	13	75	88		100							
Clostridium perfringens	1				100								
Other *Clostridium*	7		14		57	71	86				100		

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 12. *Activity of cefazaflur against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	41	2			5				10	12	42	100
Bacteroides melaninogenicus	59	46	81	85	88		93	95	98	100		
Other *Bacteroides* and *Selenomonas*	21	14	43		52	62	67		76	86	91	100
Fusobacterium nucleatum	8	75	100									
Other *Fusobacterium*	12	25	42	50			58		67		75	100
Peptococcus and *Gaffkya*	17	24	94	100								
Peptostreptococcus	15	27	87		93		100					
Anaerobic and microaerophilic streptococci	6	17	67	83								100
Gram-negative cocci	7		86						100			
Eubacterium	7	14	43				57	71	86	100		
Arachnia propionica	2		100									
Propionibacterium	4		75							100		
Actinomyces	16	31	100									
Lactobacillus	10	50	80				90			100		
Clostridium perfringens	8			38		50	88			100		
Other *Clostridium*	26	4	8	15	23	65	73	85	92	100		

[a] Includes all strains identified as subspecies of *B. fragilis*.

(1); therefore, organisms with MICs of 64 μg or more/ml would be considered resistant. Results with cefoxitin against the anaerobes indicate that the majority of strains are inhibited at an achievable level of 32 μg/ml. However, some of the groups of bacteria appear to be more resistant than those reported in a previous study (36). At that time, all *B. fragilis* strains were inhibited by 32 μg/ml, whereas only 85% of recent isolates are susceptible to that level.

Nonetheless, cefoxitin is distinctly more active than any of the cephalosporins against *B. fragilis*. Other *Fusobacterium* species, gram-positive cocci, and gram-positive, non-sporeforming bacilli also are demonstrating higher inhibitory end points. The *B. fragilis* strains in the current study appear to be less susceptible to cefoxitin than those reported by others (6, 28, 42), but the remainder of anaerobes other than *Clostridium* species appear slightly more susceptible than those reported by Tally et al. (42).

Chloramphenicol. The activity of chloramphenicol against the anaerobes tested is shown in Table 14. A level of 16 μg/ml is readily achievable, and all but two of the bacteria were inhibited at this concentration. Only one strain in the *B. fragilis* group (*B. fragilis* subsp. *thetaiotaomicron* or *Bacteroides thetaiotaomicron*)

TABLE 13. *Activity of sodium cefoxitin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	40		3	5	8	15	40	65	85	98		100
Bacteroides melaninogenicus	55	20	89	93	98		100					
Other *Bacteroides* and *Selenomonas*	21		43	48	62	71	81		86		91	100
Fusobacterium nucleatum	8	25	75		88	100						
Other *Fusobacterium*	12	8	33	42	50		75	83				100
Peptococcus and *Gaffkya*	17	29	82	88	94			100				
Peptostreptococcus	15		60	73	87	93		100				
Anaerobic and microaerophilic streptococci	6		33	50	83			100				
Gram-negative cocci	7	14	43	100								
Eubacterium	7		14	43	57	71	86		100			
Arachnia propionica	2			100								
Propionibacterium	4		75					100				
Actinomyces	16	38	88	94	100							
Lactobacillus	10		40		60	70	80	90				100
Clostridium perfringens	8		13	50	75			100				
Other *Clostridium*	26		4		19	46	54	58	62	77	100	

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 14. *Activity of chloramphenicol against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)									
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0
Bacteroides fragilis[a]	76		1		5	30	86	99	100		
Bacteroides melaninogenicus	68	10	25	56	91	100					
Other *Bacteroides* and *Selenomonas*	72	1	8	38	65	86	96	99			100
Fusobacterium nucleatum	18		72	94	100						
Other *Fusobacterium*	16		50	88	100						
Peptococcus and *Gaffkya*	59	2	3	42	88	98	100				
Peptostreptococcus	29	3	21	66	97		100				
Anaerobic and microaerophilic streptococci	10				40	80	100				
Gram-negative cocci	25		32	68	96	100					
Eubacterium	17		6	24	65	100					
Arachnia propionica	3			33	67	100					
Propionibacterium	12		50	75	92		100				
Actinomyces	16		19	81	88	94	100				
Bifidobacterium	5		20		60	100					
Lactobacillus	18		17	44	83	94		100			
Clostridium perfringens	9				22	100					
Other *Clostridium*	34		3	6	27	68	91	100			

[a] Includes all strains identified as subspecies of *B. fragilis*.

required 32 μg/ml for inhibition, and one strain of *B. clostridiiformis* subsp. *clostridiiformis* was inhibited by 128 μg/ml. These results are similar to previous reports from this laboratory and to those of others (2, 16, 18, 21, 32, 33, 39, 41, 46, 51).

Thiamphenicol, an analogue of chloramphenicol, with substitution of the *p*-nitro group by a methylsulfonyl moiety, and its parenteral form, thiamphenicol glycinate, were tested during the first part of this study. These compounds produce serum levels equivalent to those of chloramphenicol and have been found to produce less serious side effects than does chloramphenicol (46). Results obtained with thiamphenicol are given in Table 15. Thiamphenicol glycinate was equivalent to thiamphenicol in activity. Thiamphenicol was similar in activity to chloramphenicol, with most of the strains tested inhibited by 16 μg or less/ml. The organisms that required 32 μg or more/ml for inhibition were one strain each of *Bacteroides* species, *B. corrodens*, *Lactobacillus plantarum*, and *Clostridium difficile*.

Macrolides and similar drugs. Clindamycin was very active against most of the anaerobes at 8 μg or less/ml (Table 16). All *B. fragilis* strains were inhibited at this level. Strains that required 16 μg or more/ml for inhibition were three of the *Bacteroides* species, nine of the *Peptococcus* species, a *Lactobacillus catenaforme*, and four of the *Clostridium* species.

For most of the groups of anaerobes tested, results are in agreement with previously published studies (2, 17, 18, 21, 32–34, 39, 41, 47, 50, 51). With regard to resistance among the *Peptococcus*, it was not observed by Werner and Böhm (47) but was observed by Martin et al. (21) and in our own earlier studies (18). This may be an important consideration in anaerobic pleuropulmonary infections and in certain other anaerobic infections where clindamycin may be recommended when penicillin cannot be used. Resistance among *Clostridium* species other than *C. perfringens* has been noted previously (34, 41, 50). However, despite the widespread use of this compound over the past few years, resistance does not appear to be emerging among anaerobes previously susceptible to it.

The activity of erythromycin against the anaerobes tested in the second part of the study is indicated in Table 17. These results show that the *B. fragilis* strains, and the anaerobic and microaerophilic streptococci, showed more resistance to erythromycin, whereas the fusobacteria and *Clostridium* species were more susceptible than strains used in the first part of the study. These results were the subject of a previous publication (37). These disparities were not observed with clindamycin in the two parts of the study. Erythromycin was much less active than clindamycin against *B. fragilis*, the fusobacteria, and the gram-negative cocci. It showed greater activity against the anaerobic streptococci and clostridia and had similar activity against the remainder of the strains tested.

Josamycin, a macrolide structurally similar to erythromycin and with similar in vitro activity against facultative bacteria, does not induce

TABLE 15. *Activity of thiamphenicol against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)									
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0
Bacteroides fragilis[a]	42				5	21	71	100			
Bacteroides melaninogenicus	59	9	24	51	90	100					
Other *Bacteroides* and *Selenomonas*	21		19	24	57	76	86	91	95		100
Fusobacterium nucleatum	8		100								
Other *Fusobacterium*	12		42	92	100						
Peptococcus and *Gaffkya*	17			35	71	100					
Peptostreptococcus	15		33	47	93	100					
Anaerobic and microaerophilic streptococci	6				50	83		100			
Gram-negative cocci	7		43	86	100						
Eubacterium	7				43	57	100				
Arachnia propionica	2			50	100						
Propionibacterium	4		50	75			100				
Actinomyces	16		25	56	94			100			
Lactobacillus	10		10	30	50	90			100		
Clostridium perfringens	8					100					
Other *Clostridium*	27			4	22	63	78	96		100	

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 16. *Activity of clindamycin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	76	17	46	67	84	96	100					
Bacteroides melaninogenicus	64	97	100									
Other *Bacteroides* and *Selenomonas*	72	76	90	93	94		96	99				100
Fusobacterium nucleatum	18	94	100									
Other *Fusobacterium*	16	81	100									
Peptococcus and *Gaffkya*	59	41	76	80	81	83				86	93	100
Peptostreptococcus	29	52	97	100								
Anaerobic and microaerophilic streptococci	10	40	70	80	90		100					
Gram-negative cocci	25	88	100									
Eubacterium	17	41	77	88	100							
Arachnia propionica	3	33	100									
Propionibacterium	12	67	100									
Actinomyces	16	56	100									
Bifidobacterium	5	80		100								
Lactobacillus	18	56	83	89		94				100		
Clostridium perfringens	9	33	57	89	100							
Other *Clostridium*	34	9	41	53	62	79	88	91				100

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 17. *Activity of erythromycin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	34	3	6	15	24	47	71	88	94	100		
Bacteroides melaninogenicus	9	44	89	100								
Other *Bacteroides* and *Selenomonas*	51	10	45	78	86	96		98				100
Fusobacterium nucleatum	10					30	70	90		100		
Other *Fusobacterium*	4				25	50	75		100			
Peptococcus and *Gaffkya*	42		5	36	71	83						100
Peptostreptococcus	13	77	85	92	100							
Anaerobic and microaerophilic streptococci	4		100									
Gram-negative cocci	19		5	32	58	79	90	100				
Eubacterium	9	44	100									
Arachnia propionica	1			100								
Propionibacterium	8	88		100								
Bifidobacterium	5	60	100									
Lactobacillus	8	63	88									100
Clostridium perfringens	1			100								
Other *Clostridium*	7	14	71	100								

[a] Includes all strains identified as subspecies of *B. fragilis*.

macrolide resistance among staphylococci (30). It has been shown to be active against *B. fragilis* and other anaerobic bacteria (17, 20, 27, 35). The susceptibility of the anaerobes from the second part of the study is given in Table 18. On a weight basis, josamycin was more active than erythromycin or clindamycin against *B. fragilis* and showed activity similar to that of erythromycin with the remainder of the anaerobes. It, like erythromycin, was more active than clindamycin against the clostridia but was less active than clindamycin against the fusobacteria. It appears to be as active as rosamicin against *B. fragilis* (37) and other anaerobes. However, a direct comparison with the same strains was not made.

A new oligosaccharide, everninomicin B, was tested and the results are shown in Table 19. Pharmacological data are not yet available, but on a weight basis everninomicin is less active

TABLE 18. *Activity of josamycin against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	34		29	53	77	100						
Bacteroides melaninogenicus	9	56	100									
Other *Bacteroides* and *Selenomonas*	51	12	90	92	98							100
Fusobacterium nucleatum	10					30	80	100				
Other *Fusobacterium*	4				25			50	75	100		
Peptococcus and *Gaffkya*	44		43	82	84		87		93	98	100	
Peptostreptococcus	13	31	85	92								100
Anaerobic and microaerophilic streptococci	4		50	75	100							
Gram-negative cocci	19		5	32	42	68	84	95			100	
Eubacterium	9	33	78	100								
Arachnia propionica	1			100								
Propionibacterium	8	38	100									
Bifidobacterium	5		100									
Lactobacillus	8	38	75	88					100			
Clostridium perfringens	1				100							
Other *Clostridium*	7	14	71	100								

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 19. *Activity of everninomicin B against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	36			3	6	14	25	42	69	94	97	100
Bacteroides melaninogenicus	58	9	64	78	85	97	100					
Other *Bacteroides* and *Selenomonas*	20			15	40	45	55	65	70	95	100	
Fusobacterium nucleatum	8						50	88		100		
Other *Fusobacterium*	10							40	50			100
Peptococcus and *Gaffkya*	13	69	92									100
Peptostreptococcus	12	50	100									
Anaerobic and microaerophilic streptococci	5	80	100									
Gram-negative cocci	6						33	67	83	100		
Eubacterium	7	43	86	100								
Arachnia propionica	2										50	100
Propionibacterium	4		25									100
Actinomyces	16	69	94	100								
Lactobacillus	7	86	100									
Clostridium perfringens	7	43	71		86	100						
Other *Clostridium*	23	13	83	91	100							

[a] Includes all strains identified as subspecies of *B. fragilis*.

than clindamycin against *B. fragilis* and other gram-negative anaerobes and more active against the clostridia, including strains resistant to clindamycin. It was also more active than was clindamycin against gram-positive, non-sporeforming bacteria, with the exception of the few strains of *Arachnia*, *Propionibacterium*, and *Actinomyces* tested.

Tetracyclines. The activities of tetracycline, doxycycline, and minocycline were determined throughout the study. Results are shown in Tables 20 through 22. Only 42% of strains in the *B. fragilis* group remain susceptible to tetracycline at 4 μg or less/ml and 46% at 8 μg or less/ml. Other groups of anaerobes also exhibited less suceptibility to tetracycline than in prior reports (16, 33, 38). The current results are similar to other recently published data (2, 9, 21, 32, 41, 51). Doxycycline remains more active than tetracycline against anaerobes but is

TABLE 20. *Activity of tetracycline against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)									
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0
Bacteroides fragilis[a]	76		25	40		42	46	68	93	99	100
Bacteroides melaninogenicus	67	51	75	76	79	87	94	96	99	100	
Other *Bacteroides* and *Selenomonas*	72	8	33	35	43	50	60	75	93	99	100
Fusobacterium nucleatum	18	33	100								
Other *Fusobacterium*	16	19	88	94				100			
Peptococcus and *Gaffkya*	59	2	25	29	36		37	61	92	100	
Peptostreptococcus	29	28	38	41	48	52	72	86	97	100	
Anaerobic and microaerophilic streptococci	10		50	60	70	90			100		
Gram-negative cocci	26	8	54	69	73			85	92	100	
Eubacterium	17	6	24	59	65		77	82	94	100	
Arachnia propionica	3		67							100	
Propionibacterium	12		58	75	83				100		
Actinomyces	16		56	69	94			100			
Bifidobacterium	5				60				100		
Lactobacillus	17	6	24	53	59	65	77	82	100		
Clostridium perfringens	9	11	22		56	67		78		100	
Other *Clostridium*	33	6	36	46	49	52	61	67	76	91	100

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 21. *Activity of doxycycline against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)									
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0
Bacteroides fragilis[a]	76	5	41	42	50	75	88	97	100		
Bacteroides melaninogenicus	67	66	75	78	90	96	97	99		100	
Other *Bacteroides* and *Selenomonas*	72	15	40	43	53	68	79	85	96	99	100
Fusobacterium nucleatum	18	44	100								
Other *Fusobacterium*	16	38	88				100				
Peptococcus and *Gaffkya*	60	8	28	35	40	70	93	98	100		
Peptostreptococcus	29	31	45		66	79	97	100			
Anaerobic and microaerophilic streptococci	10	20	70	90			100				
Gram-negative cocci	26	4	58	69	73	81	96	100			
Eubacterium	17	12	59	65	77	82	88	100			
Arachnia propionica	3		67				100				
Propionibacterium	12	17	75	83		92			100		
Actinomyces	16		63	69	94	100					
Bifidobacterium	5			20	40	60		100			
Lactobacillus	17	12	35	59	71	82	94	100			
Clostridium perfringens	9	11	67			78	89	100			
Other *Clostridium*	33	21	49	52	61	68	82	97		100	

[a] Includes all strains identified as subspecies of *Bacteroides fragilis*.

slightly less active than minocycline against the same strains, except for *C. perfringens*.

Metronidazole. The susceptibility of the anaerobes to metronidazole is shown in Table 23. With the exception of the gram-positive, nonsporeforming bacilli, the majority of strains were inhibited by 16 μg or less/ml. Bacteria requiring MICs of 32 μg or more/ml were two strains of *Bacteroides pneumosintes*, one *Peptococcus saccharolyticus*, one *Peptostreptococcus* species, three *Streptococcus intermedius*, two *Eubacterium* species, two *Arachnia propionica*, eight *Propionibacterium acnes*, two *Propionibacterium avidum*, eight *Actinomyces* species, one *Actinomyces israelii*, one *Actinomyces viscosus*, and one each of *Bifidobacterium* species, *Bifidobacterium adolescentis* var. B, and *Bifidobacterium infantis*. These results generally agree with those in previous publications (11, 19, 23, 31, 34, 41, 44, 45). We did not observe

TABLE 22. *Activity of minocycline against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)							
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0
Bacteroides fragilis[a]	76	29	49	57	70	82	97	100	
Bacteroides melaninogenicus	65	71	74	82	89	97	99	100	
Other *Bacteroides* and *Selenomonas*	70	29	49	56	70	79	90	96	100
Fusobacterium nucleatum	18	67	100						
Other *Fusobacterium*	16	69	94			100			
Peptococcus and *Gaffkya*	58	19	43	59	74	93	97	100	
Peptostreptococcus	28	39	54	75	82	93	100		
Anaerobic and microaerophilic streptococci	10	40	90			100			
Gram-negative cocci	25	12	68	72		92	100		
Eubacterium	16	25	69		81		94	100	
Arachnia propionica	3	33	100						
Propionibacterium	12	42	83		92			100	
Actinomyces	16		88		94	100			
Bifidobacterium	5	40	80		100				
Lactobacillus	17	24	59	71	82	88	100		
Clostridium perfringens	9	22	67					78	100
Other *Clostridium*	33	27	58	61	73	76	97		100

[a] Includes all strains identified as subspecies of *B. fragilis*.

TABLE 23. *Activity of metronidazole against anaerobic bacteria*

Bacteria	No. of strains tested	Cumulative % susceptible to indicated concn (μg/ml)										
		≤0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128.0	≥256
Bacteroides fragilis[a]	76	1	12	28	57	86	99	100				
Bacteroides melaninogenicus	69	15	81	93	99	100						
Other *Bacteroides* and *Selenomonas*	71	6	42	69	86	94	97		99			100
Fusobacterium nucleatum	18	56	94			100						
Other *Fusobacterium*	16	38	94	100								
Peptococcus and *Gaffkya*	58	3	72	88	98						100	
Peptostreptococcus	29	21	69	76	83	86	93	97				100
Anaerobic and microaerophilic streptococci	10		30			40					50	100
Gram-negative cocci	25	4	60	92	96	100						
Eubacterium	16	6	44	81	88							100
Arachnia propionica	3		33									100
Propionibacterium	12		8			17						100
Actinomyces	16					13		19	50	56	63	100
Bifidobacterium	5					20		40	60	80		100
Lactobacillus	19	11	37	58		68	79			84	90	100
Clostridium perfringens	9		33	78	100							
Other *Clostridium*	32	9	53	81	97	100						

[a] Includes all strains identified as subspecies of *B. fragilis*.

resistant strains of *B. fragilis*, *Fusobacterium* species, or *Clostridium* species, as was reported by Chow et al. (10).

Although all of the strains in the current study were defined as anaerobes because on initial isolation they grew poorly or not at all in the presence of air, some became more aerotolerant through subsequent in vitro cultivation. For the most part, these were the strains showing the greatest resistance to metronidazole. They belonged in the genus *Streptococcus* and in the various genera of gram-positive, non-sporeforming bacilli. However, the majority of anaerobic bacteria most frequently encountered in infections remain susceptible to achievable levels of metronidazole.

ACKNOWLEDGMENTS

We gratefully acknowledge the contribution of Godfrey K. M. Harding for his studies on identification of subspecies of the *B. melaninogenicus* strains. We thank Lubna Afzal Kureshi and Y. Y. Kwok for their excellent technical assistance and Edward O. Warburton and others at the Biomedical Engineering and Computer Center, Sepulveda VA Hospital, for their excellent assistance in compiling the data.

These studies were supported by grants from Beecham-Massengill Pharmaceuticals, Bristol, Tenn., Bristol Laboratories, Syracuse, N.Y., Delbay Pharmaceuticals, Inc., Bloomfield, N.J., E. I. Dupont de Nemours and Co., Newark, Del., Lederle Laboratories, Pearl River, N.Y., Eli Lilly and Co., Indianapolis, Ind., Pfizer Laboratories, New York, N.Y., Schering Corp., Bloomfield, N.J., G. D. Searle and Co., Chicago, Ill., Smith, Kline and French Laboratories, Philadelphia, Pa., and Zambon, S.p.A., Bresso, Milano, Italy.

LITERATURE CITED

1. **Actor, P., J. V. Uri, J. R. Guarini, I. Zajac, L. Phillips, C. S. Sachs, R. M. De Marinis, J. R. E. Hoover, and J. A. Weisbach.** 1975. A new parenteral cephalosporin. SK&F 59962: *in vitro* and *in vivo* antibacterial activity and serum levels in experimental animals. J. Antibiot. 28:471-476.
2. **Blazevic, D. J.** 1976. Antibiotic susceptibility of the subspecies of *Bacteroides fragilis*. Antimicrob. Agents Chemother. 9:481-484.
3. **Blazevic, D. J., and J. M. Matsen.** 1974. Susceptibility of anaerobic bacteria to carbenicillin. Antimicrob. Agents Chemother. 5:462-465.
4. **Bodey, G. P., V. Rodriguez, N. Horikoshi, and M. Valdivieso.** 1973. New antibiotics of potential importance for immunosuppressed patients. Transplant. Proc. 5(Suppl.):1227-1231.
5. **Bodey, G. P., and S. Weaver.** 1976. In vitro studies of cefamandole. Antimicrob. Agents Chemother. 9:452-457.
6. **Brumfitt, W., J. Kosmides, J. M. T. Hamilton-Miller, and J. N. G. Gilchrist.** 1974. Cefoxitin and cephalothin: antimicrobial activity, human pharmacokinetics, and toxicology. Antimicrob. Agents Chemother. 6:290-299.
7. **Cato, E. P., and J. L. Johnson.** 1976. Reinstatement of species rank for *Bacteroides fragilis, B. ovatus, B. distasonis, B. thetaiotaomicron*, and *B. vulgatus*: designation of neotype strains for *Bacteroides fragilis* (Veillon and Zuber) Castellani and Chalmers and *Bacteroides thetaiotaomicron* (Distaso) Castellani and Chalmers. Int. J. Syst. Bacteriol. 26:230-237.
8. **Cato, E. P., and C. W. Salmon.** 1976. Transfer of *Bacteroides clostridiiformis* subsp. *clostridiiformis* (Burri and Ankersmit) Holdeman and Moore and *Bacteroides clostridiiformis* subsp. *girans* (Prévot) Holdeman and Moore to the genus *Clostridium* as *Clostridium clostridiiforme* (Burri and Ankersmit) comb. nov.: emendation of description and designation of neotype strain. Int. J. Syst. Bacteriol. 26:205-211.
9. **Chow, A. W., V. Patten, and L. B. Guze.** 1975. Comparative susceptibility of anaerobic bacteria to minocycline, doxycycline, and tetracycline. Antimicrob. Agents Chemother. 7:46-49.
10. **Chow, A. W., V. Patten, and L. B. Guze.** 1975. Susceptibility of anaerobic bacteria to metronidazole: relative resistance of non-spore-forming gram-positive bacilli. J. Infect. Dis. 131:182-185.
11. **Dornbusch, K., and C. E. Nord.** 1974. *In vitro* effect of metronidazole and tinidazole on anaerobic bacteria. Med. Microbiol. Immunol. 160:265-267.
12. **Ernst, E. C., S. Berger, M. Barza, N. V. Jacobus, and F. P. Tally.** 1976. Activity of cefamandole and other cephalosporins against aerobic and anaerobic bacteria. Antimicrob. Agents Chemother. 9:852-855.
13. **Holdeman, L. V., and W. E. C. Moore (ed).** 1972. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University, Blacksburg.
14. **Jones, R. N., and P. C. Fuchs.** 1976. Identification and antimicrobial susceptibility of 250 *Bacteroides fragilis* subspecies tested by broth microdilution methods. Antimicrob. Agents Chemother. 9:719-721.
15. **Kaneuchi, C., K. Watanabe, A. Terada, Y. Benno, and T. Mitsuoka.** 1976. Taxonomic study of *Bacteroides clostridiiformis* subsp. *clostridiiformis* (Burri and Ankersmit) Holdeman and Moore and of related organisms: proposal of *Clostridium clostridiiformis* (Burri and Ankersmit) comb. nov. and *Clostridium symbiosum* (Stevens) comb. nov. Int. J. Syst. Bacteriol. 26:195-204.
16. **Kislak, J. W.** 1972. The susceptibility of *Bacteroides fragilis* to 24 antibotics. J. Infect. Dis. 125:295-299.
17. **Kosakai, N., J. Igari, and T. Oguri.** 1969. Susceptibility of recently isolated pathogens to josamycin, erythromycin and lincomycin. Chemotherapy (Tokyo) 17:572-575.
18. **Kwok, Y. Y., F. P. Tally, V. L. Sutter, and S. M. Finegold.** 1975. Disk susceptibility testing of slow-growing anaerobic bacteria. Antimicrob. Agents Chemother. 7:1-7.
19. **Lerner, P. I.** 1974. Susceptibility of pathogenic actinomycetes to antimicrobial compounds. Antimicrob. Agents Chemother. 5:302-309.
20. **Long, S. S., S. Mueller, and R. M. Swenson.** 1976. In vitro susceptibilities of anaerobic bacteria to josamycin. Antimicrob. Agents Chemother. 9:859-860.
21. **Martin, W. J., M. Gardner, and J. A. Washington II.** 1972. In vitro antimicrobial susceptibility of anaerobic bacteria isolated from clinical specimens. Antimicrob. Agents Chemother. 1:148-158.
22. **Meyers, B. R., B. Leng, and S. Z. Hirshman.** 1975. Cefamandole: antimicrobial activity in vitro of a new cephalosoporin. Antimicrob. Agents Chemother. 8:737-741.
23. **Nastro, L. J., and S. M. Finegold.** 1972. Bactericidal activity of five antimicrobial agents against *Bacteroides fragilis*. J. Infect. Dis. 126:104-107.
24. **Neu, H. C.** 1974. Antimicrobial activity and human pharmacology of amoxicillin. J. Infect. Dis. 129(Suppl.):S123-S131.
25. **Neu, H. C.** 1974. Cefamandole, a cephalosporin antibiotic with an unusually wide spectrum of activity. Antimicrob. Agents Chemother. 6:177-182.
26. **Neu, H. C., and G. J. Garvey.** 1975. Comparative in vitro activity and clinical pharmacology of ticarcillin and carbenicillin. Antimicrob. Agents Chemother. 8:457-462.
27. **Ninomiya, K., K. Watanabe, K. Ueno, S. Suzuki, I. Mochizuki, Y. Simizu, T. Isogai, and T. Nisiura.** 1974. Susceptibility of anaerobic bacteria to SF 837. Chemotherapy (Tokyo) 22:1058-1064.
28. **Norrby, R., J. E. Brorsson, and S. Seeberg.** 1976. Comparative study of the in vitro antibacterial activity of cefoxitin, cefuroxime, and cephaloridine. Antimicrob. Agents Chemother. 9:506-510.
29. **Olsson, B., C. E. Nord, and T. Wadström.** 1976. Formation of beta-lactamase in *Bacteroides fragilis*: cell bound and extracellular activity. Antimicrob. Agents Chemother. 9:727-735.
30. **Osono, T., and H. Umezawa.** 1971. Josamycin, a new macrolide antibiotic of resistance non-inducing type, p. 41-120. *In* S. Mitsuhashi (ed.), Drug action and drug resistance in bacteria. University Park Press, Baltimore.
31. **Reynolds, A. V., J. M. T. Hamilton-Miller, and W. Brumfitt.** 1975. A comparison of the *in vitro* activity of metronidazole, tinidazole, and nimorazole against gram-negative anaerobic bacteria. J. Clin. Pathol. 28:775-778.
32. **Rotilie, C. A., R. J. Fass, R. B. Prior, and R. L. Perkins.** 1975. Microdilution technique for antimicrobial susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. 7:311-315.
33. **Sapico, F. L., Y. Y. Kwok, V. L. Sutter, and S. M. Finegold.** 1972. Standardized antimicrobial disc susceptibility testing of anaerobic bacteria: in vitro sus-

ceptibility of *Clostridium perfringens* to nine antibiotics. Antimicrob. Agents Chemother. 2:320-325.
34. **Staneck, J. L., and J. A. Washington II.** 1974. Antimicrobial susceptibilities of anaerobic bacteria: recent clinical isolates. Antimicrob. Agents Chemother. 6:311-315.
35. **Strausbaugh, L. J., J. A. Dilworth, J. M. Gwaltney, Jr., and M. A. Sande.** 1976. In vitro susceptibility studies with josamycin and erythromycin. Antimicrob. Agents Chemother. 9:546-548.
36. **Sutter, V. L., and S. M. Finegold.** 1975. Susceptibility of anaerobic bacteria to carbenicillin, cefoxitin and related drugs. J. Infect. Dis. 131:417-422.
37. **Sutter, V. L., and S. M. Finegold.** 1976. Rosamicin: in vitro activity against anaerobes and comparison with erythromycin. Antimicrob. Agents Chemother. 9:350-351.
38. **Sutter, V. L., Y. Y. Kwok, and S. M. Finegold.** 1972. Standardized antimicrobial disc susceptibility testing of anaerobic bacteria. I. Susceptibility of *Bacteroides fragilis* to tetracycline. Appl. Microbiol. 23:268-275.
39. **Sutter, V. L., Y. Y. Kwok, and S. M. Finegold.** 1973. Susceptibility of *Bacteroides fragilis* to six antibiotics determined by standardized antimicrobial disc susceptibility testing. Antimicrob. Agents Chemother. 3:188-193.
40. **Sutter, V. L., V. L. Vargo, and S. M. Finegold.** 1975. Wadsworth anaerobic bacteriology manual, 2nd ed. University of California, Los Angeles Extension Division, Los Angeles.
41. **Tally, F. P., A. Y. Armfield, V. R. Dowell, Jr., Y. Y. Kwok, V. L. Sutter, and S. M. Finegold.** 1974. Susceptibility of *Clostridium ramosum* to antimicrobial agents. Antimicrob. Agents Chemother. 5:589-593.
42. **Tally, F. P., N. V. Jacobus, J. G. Bartlett, and S. L. Gorbach.** 1975. Susceptibility of anaerobes to cefoxitin and other cephalosporins. Antimicrob. Agents Chemother. 7:128-132.
43. **Tally, F. P., N. V. Jacobus, J. G. Bartlett, and S. L. Gorbach.** 1975. In vitro activity of penicillins against anaerobes. Antimicrob. Agents Chemother. 7:413-414.
44. **Tally, F. P., V. L. Sutter, and S. M. Finegold.** 1972. Metronidazole versus anaerobes. In vitro data and initial clinical observations. Calif. Med. 117:22-26.
45. **Ueno, K., K. Ninomiya, and S. Suzuki.** 1971. Antibacterial activity of metronidazole against anaerobic bacteria. Chemotherapy (Tokyo) 19:111-114.
46. **Van Beers, D., E. Schoutens, M. P. Vanderlinden, and E. Yourassowsky.** 1975. Comparative in vitro activity of chloramphenicol and thiamphenicol on common aerobic and anaerobic Gram-negative bacilli (Salmonella and Shigella excluded). Chemotherapy (Basel) 21:73-81.
47. **Werner, H., and G. Böhm.** 1974. Susceptibility of nonsporing anaerobes of the genera *Bacteroides, Fusobacterium, Sphaerophorus, Peptococcus* and *Peptostreptococcus* to clindamycin. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 229:401-408.
48. **Werner, H., G. Rintelen, and H. Kunstek-Santos.** 1975. A new butyric acid-producing *Bacteroides* species: *B. splanchnicus* n. sp. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 231:133-144.
49. **Wick, W. E., and D. A. Preston.** 1972. Biological properties of three 3-heterocyclic-thiomethyl cephalosporin antibiotics. Antimicrob. Agents Chemother. 1:221-234.
50. **Wilkins, T. D., and T. Thiel.** 1973. Resistance of some species of *Clostridium* to clindamycin. Antimicrob. Agents Chemother. 3:136-137.
51. **Zabransky, R. J., J. A. Johnston, and K. J. Hauser.** 1973. Bacteriostatic and bactericidal activities of various antibiotics against *Bacteroides fragilis*. Antimicrob. Agents Chemother. 3:152-156.