SUSCEPTIBILITY OF ANAEROBIC BACTERIA TO BETA-LACTAM ANTIBIOTICS AND BETA-LACTAMASE PRODUCTION

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SUMMARY. We examined the susceptibility of various anaerobes to four beta-lactamase susceptible (ampicillin, amoxycillin, cyclacillin, and penicillin G) and two beta-lactamase resistant (moxalactam, and N-F-thienamycin) beta-lactam antibiotics and measured beta-lactamase production. Members of the *Bacteroides* groups were most resistant to the six antibiotics. N-F-thienamycin was the most effective antimicrobial agent against all the test strains, moxalactam the next most effective, and penicillin G the least. Beta-lactamase production was mainly confined to *Bacteroides* species. Cephalosporinase was the most common beta-lactamase produced; penicillinase was detected less often. About two thirds of the penicillin-resistant isolates produced cephalosporinase. N-F-thienamycin and moxalactam were the most active agents against those anaerobes that were resistant to many beta-lactam antibiotics.

INTRODUCTION

During the last two decades the clinical significance of anaerobic bacteria has been recognised. Infections of almost all body sites have been described and this has stimulated interest in the causative role of these organisms. The major factor contributing to this increased interest has been the increased ease with which the more common pathogenic anaerobes can be isolated and identified.

In the past, most reports have indicated that anaerobic isolates other than certain members of the *Bacteroides fragilis* group are, in general, susceptible to many antibiotics (Finegold and Hewitt, 1956; Ingham *et al.*, 1968; Bodner *et al.*, 1972; Martin, Gardner and Washington, 1972; Mitchell, 1973; Tally *et al.*, 1975). However, more recent reports have indicated an increased resistance of anaerobes to certain antibiotics. Several studies have shown an increase in resistance of *B. fragilis* to tetracycline (Nastro and Finegold, 1972; Mitchell, 1973; Zabransky, Johnston and Hauser, 1973), minocycline and clindamycin (Staneck and Washington, 1974; Bawdon *et al.*, 1979). Moreover, increased resistance to clindamycin and penicillin G has been noted among *Clostridium* spp. (Staneck and Washington, 1974; Marrie *et al.*, 1981). This resistance can be the result of enzymatic inactivation of the antibiotic or decreased

permeability of the cell to the antibiotic. New beta-lactamase resistant beta-lactam antibiotics have been developed to overcome the enzymatic types of resistance.

We examined the susceptibility of a wide variety of anaerobes to four beta-lactamase susceptible (ampicillin, amoxycillin, cyclacillin and penicillin G) and two beta-lactamase resistant (moxalactam and N-F-thienamycin) beta-lactam antibiotics, examined beta-lactamase production by resistant strains and attempted to correlate beta-lactamase production with resistance to penicillin G.

MATERIALS AND METHODS

Antibiotics. The following antibiotic reference powders were provided by the manufacturers: ampicillin and amoxycillin (Beecham Laboratories, Bristol, TN.); cyclacillin (Wyeth Laboratories, Philadelphia, PA.); moxalactam and penicillin G (Eli Lilly Laboratories, Indianapolis, IN.); and N-formimidoyl thienamycin (Merck, Sharp and Dohme Laboratories, West Point, PA.). The powders were stored at -20°C and working solutions were prepared and incorporated in media on the same day.

Organisms. One hundred and seventy nine strains of anaerobic bacteria from the stock collection of the Division of Infectious Diseases at LSU Medical Center were used. Each strain was grown on prereduced Brucella agar with vitamin K ($0.1\text{ }\mu\text{g/ml}$) and sheep's blood 5%. Growth was then transferred to thioglycollate broth by sterile cotton swabs and the turbidity adjusted to be equivalent to a 0.5 MacFarland standard.

MIC determinations. Serial twofold dilutions ($64\text{--}0.125\text{ }\mu\text{g/ml}$) of each antibiotic were prepared in Brucella agar with vitamin K ($0.1\text{ }\mu\text{g/ml}$) and sheep's blood 5%. All plates were prereduced by holding them in anaerobic conditions overnight before seeding. Each organism was seeded in duplicate on to the plates by a Steers' replicator delivering 0.005 ml of the thioglycollate broth suspension on to the surface of each plate. The final inoculum was approximately 10^5 cfu . The plates were placed in an anaerobic chamber at 35°C for 48 h. The MIC was the lowest concentration of each antibiotic that inhibited visible growth of the organism (Greenberg *et al.*, 1979).

Beta-lactamase assays. Each organism was tested by two methods:

1. A vial containing penicillin G 20×10^6 units was diluted to a concentration of 10^6 units/ml with phosphate buffer ($\text{pH } 7.0$) containing phenol red indicator 0.1% . The pH of the solution was then raised to 8.5 with 0.1 N NaOH . Three drops of solution were added to each well of a microtitration tray and seeded with several colonies of an isolate. After incubation for 1 h at room temperature the presence of penicillinase was indicated by a change of colour from red-purple to yellow.

2. Chromogenic cephalosporin compound 87/312 (Glaxo Inc., Ft. Lauderdale, FL) 5 mg was dissolved in 1 ml of dimethylsulfoxide and diluted with 19 ml of phosphate buffer ($\text{pH } 7.0$). Three drops of this solution were added to each well in a microtitration tray and seeded with several colonies of an isolate. After incubation for 1 h at room temperature, the presence of cephalosporinase was indicated by a change of colour from yellow to rust-red.

RESULTS

Sensitivity of anaerobes to beta-lactam antibiotics

The results of the MIC determinations with six beta-lactam antibiotics are shown in table I. *Bacteroides* spp. were the most resistant group. We chose an MIC of $16\text{ }\mu\text{g/ml}$ as the breakpoint for susceptibility. N-F-thienamycin was the most active antibiotic against the 40 test strains of the *B. fragilis* group (ss. *fragilis*, ss. *vulgatus*, ss. *distasonis*, and ss. *thetaiotaomicron*); 93% of the strains were inhibited by $4\text{ }\mu\text{g/ml}$ and

TABLE I
The susceptibility of anaerobic bacteria to six beta-lactam antibiotics

Species	Number of strains	Mean MIC values ($\mu\text{g/ml}$) of the six antibiotics for the test strains (range of results)					
		Ampicillin	Amoxycillin	Cyclacillin	Moxalactam	Penicillin	N-F-Thienamycin
<i>B. fragilis</i>	10	32 (0.25- > 64)	32 (≤ 0.125 - > 64)	64 (2- > 64)	4 (0.5-32)	32 (8- > 64)	1 (≤ 0.125 -16)
<i>B. vulgatus</i>	10	64 (16- > 64)	64 (16- > 64)	64 (16- > 64)	8 (1-64)	32 (8- > 64)	1 (0.25-16)
<i>B. distasonis</i>	10	32 (8-64)	32 (4-64)	32 (16- > 64)	32 (2- > 64)	8 (≤ 0.25 - > 64)	0.5 (0.25-1)
<i>B. thetaiotaomicron</i>	10	32 (16-64)	64 (16- > 64)	64 (32-64)	64 (4- > 64)	16 (≤ 0.25 - > 64)	1 (1-2)
<i>B. melaninogenicus</i>	11	4 (≤ 0.125 -64)	2 (≤ 0.125 -64)	8 (0.25-64)	4 (0.125-32)	8 (≤ 0.25 - > 64)	0.125 (≤ 0.125 -0.5)
<i>B. melaninogenicus</i>	9	0.25 (≤ 0.125 -32)	0.25 (≤ 0.125 -64)	1 (0.25-32)	0.5 (≤ 0.125 -16)	4 (≤ 0.25 - > 64)	0.125 (≤ 0.125 -0.25)
<i>B. asaccharolyticus</i>	11	2 (≤ 0.125 - > 64)	2 (≤ 0.125 - > 64)	4 (≤ 0.5 -32)	2 (≤ 0.125 -16)	8 (≤ 0.25 - > 64)	0.125 (≤ 0.125 -0.125)
<i>Bacteroides</i> spp.	2	≤ 0.125	≤ 0.125	0.125	0.125	≤ 0.125	≤ 0.125
<i>F. nucleatum</i>	5	0.125 (≤ 0.125 -4)	0.125 (≤ 0.125 -0.25)	0.5 (≤ 0.125 -8)	0.5 (≤ 0.125 -8)	4 (≤ 0.25 - > 64)	0.125 (≤ 0.125 -1)
<i>F. mortiferum</i>	5	8 (0.25- > 64)	8 (0.25- > 64)	16 (1- > 64)	8 (1-32)	16 (2- > 64)	1 (0.25-4)
<i>F. necrophorum</i>	4	≤ 0.125	≤ 0.125	0.5 (0.25-0.5)	≤ 0.125	≤ 0.25	≤ 0.125
<i>Fusobacterium</i> spp.	5	≤ 0.125	≤ 0.125	0.25 (≤ 0.125 -0.5)	0.125 (≤ 0.125 -1)	0.5 (≤ 0.25 -8)	≤ 0.125
<i>Peptost. anaerobius</i>	20	0.125 (≤ 0.125 -8)	0.125 (≤ 0.125 -16)	2 (≤ 0.125 -64)	2 (0.5-64)	2 (≤ 0.25 - > 64)	0.125 (≤ 0.125 -1)
<i>Peptost. micros</i>	5	≤ 0.125	≤ 0.125	0.5 (0.25-2)	0.25 (≤ 0.125 -1)	≤ 0.25	≤ 0.125
<i>Pept. variabilis</i>	5	0.125 (≤ 0.125 -0.25)	0.125 (≤ 0.125 -0.25)	2 (1-4)	1 (0.25-2)	≤ 0.25	0.125 (0.125-0.25)
<i>Pept. magnus</i>	5	0.125 (≤ 0.125 -0.25)	0.25	2	2 (1-2)	≤ 0.25 (≤ 0.25 -1)	0.125
<i>Pept. morbillorum</i>	4	≤ 0.125	≤ 0.125	0.25 (≤ 0.125 -0.5)	0.125 (≤ 0.125 -0.25)	≤ 0.25	≤ 0.125 (≤ 0.125 -0.125)
<i>Pept. asaccharolyticus</i>	5	≤ 0.125	≤ 0.125	0.125 (≤ 0.125 -2)	0.125 (≤ 0.125 -0.25)	0.5	≤ 0.125
<i>Pept. prevoti</i>	6	≤ 0.125	≤ 0.125 (≤ 0.125 -0.25)	0.125 (≤ 0.125 -1)	0.25 (≤ 0.125 -2)	0.5 (≤ 0.25 -2)	≤ 0.125 (≤ 0.125 -0.125)
<i>C. perfringens</i>	10	0.125 (≤ 0.125 -0.25)	≤ 0.125	0.25 (0.25-0.5)	1 (0.5-2)	0.5 (≤ 0.25 -8)	0.25
<i>Clostridium</i> spp.	10	0.25 (≤ 0.125 -16)	0.25 (≤ 0.125 -32)	2 (≤ 0.125 -16)	4 (≤ 0.125 - > 64)	0.25 (≤ 0.125 -16)	0.5 (≤ 0.125 -4)
<i>E. lentum</i>	5	0.125 (≤ 0.125 -4)	0.125 (≤ 0.125 -2)	0.5 (≤ 0.125 -4)	0.5 (≤ 0.125 -8)	0.25 (≤ 0.125 -0.5)	≤ 0.125
<i>Prop. acnes</i>	9	0.125 (≤ 0.125 -2)	0.125 (≤ 0.125 -2)	1 (≤ 0.125 -32)	2 (0.5-32)	0.125 (≤ 0.125 -4)	≤ 0.125
<i>Bifidobacterium</i> spp.	3	≤ 0.125	≤ 0.125	0.5 (0.25-1)	0.125 (≤ 0.125 -0.5)	≤ 0.125	≤ 0.125

all of them by 16 µg/ml. Moxalactam was the second most active antibiotic against this group; 63% of the strains were inhibited by 16 µg/ml. The other group of *Bacteroides* strains that showed some resistance was the pigmented group—*B. melaninogenicus* ss. *melaninogenicus* and ss. *intermedius* and *B. asaccharolyticus*. N-F-thienamycin and moxalactam were again the most active antibiotics against the 31 strains tested. N-F-thienamycin 0.5 µg/ml inhibited all the strains and with moxalactam 61% of the strains were inhibited by 4 µg/ml and all by 16 µg/ml. Ampicillin, amoxycillin, cyclacillin and penicillin G were less effective; 16 µg/ml inhibited 71, 77, 81 and 58% respectively of the strains.

In the *Fusobacterium* group, resistance to all the beta-lactams was low; strains of *F. mortiferum* were the most resistant and *F. necrophorum* the most susceptible. N-F-thienamycin 4 µg/ml inhibited all strains and moxalactam 16 µg/ml inhibited 95%. Ampicillin, amoxycillin and cyclacillin 16 µg/ml inhibited 89% of the strains and penicillin G 16 µg/ml inhibited 85%.

Ampicillin, amoxycillin, moxalactam and N-F-thienamycin inhibited all strains of *Peptostreptococcus* at concentrations < 16 µg/ml, and cyclacillin and penicillin G inhibited 88 and 80% respectively. With all the antibiotics tested all strains of *Peptococcus* were sensitive to < 8 µg/ml. Against the anaerobic gram-positive rods (*Clostridium*, *Eubacterium*, *Propionibacterium* and *Bifidobacterium*), N-F-thienamycin was the most effective antibiotic; all strains were inhibited by 16 µg/ml. Amoxycillin, moxalactam and cyclacillin 16 µg/ml inhibited > 90% of strains.

Beta-lactamase assays

In most instances the beta-lactamase assays provided clear end points. Those that were not distinct were assessed by comparison with positive and negative controls.

Table II shows the results of the beta-lactamase assays. The *Bacteroides* strains gave the greatest number of positive results for both penicillinase and cephalosporinase. The only strains that produced penicillinase were *Bacteroides* spp. Cephalosporinase was the predominant beta-lactamase produced whereas penicillinase was often not produced. Cephalosporinase activity was strong in the producer strains; most positive results were evident within 15 min. However, even when production was detected penicillinase activity was weaker; often incubation for 45 min or more was needed for a change in the colour of the reaction mixture to appear. Cephalosporinase was produced by 87% of *B. fragilis* and 42% of the pigmented *Bacteroides* strains but only 5% of *B. fragilis* and 16% of the pigmented strains produced penicillinase. No other anaerobic species produced penicillinase and only a few strains of *Fusobacterium*, *Clostridium* and *Eubacterium* produced cephalosporinase.

For comparison of beta-lactamase production with resistance to penicillin G, organisms with MICs ≥ 16 µg/ml were considered resistant. Table III shows the number of penicillin G-resistant strains that produced beta-lactamases; 20% produced penicillinase only, 63% produced cephalosporinase only, and 20% produced both. The resistant *Bacteroides* strains were the most active producers of beta-lactamases. In the *B. fragilis* group, 85% of strains produced cephalosporinase but only 8% produced penicillinase whereas cephalosporinase was produced by 80% and penicillinase by 64% of the resistant pigmented strains. None of the remaining strains of penicillin-resistant anaerobes produced either penicillinase or cephalosporinase.

TABLE II
Production of penicillinase and cephalosporinase

Species	Number of strains	Percentage of strains that produced	
		Penicillinase	Cephalosporinase
<i>B. fragilis</i>	10	10	70
<i>B. vulgatus</i>	10	0	90
<i>B. distasonis</i>	10	10	90
<i>B. thetaiotaomicron</i>	10	0	100
<i>B. melaninogenicus</i> ss. <i>mel.</i>	11	36	55
<i>B. mel.</i> ss. <i>intermedius</i>	9	11	22
<i>B. asaccharolyticus</i>	11	36	36
<i>Bacteroides</i> spp.	2	0	100
<i>F. nucleatum</i>	5	0	0
<i>F. mortiferum</i>	5	0	0
<i>F. necrophorum</i>	4	0	0
<i>Fusobacterium</i> spp.	5	0	20
<i>Peptostr. anaerobius</i>	20	0	0
<i>Peptostr. micros</i>	5	0	0
<i>Pept. variabilis</i>	5	0	0
<i>Pept. magnus</i>	5	0	0
<i>Pept. morbillorum</i>	4	0	0
<i>Pept. asaccharolyticus</i>	5	0	0
<i>Pept. prevoti</i>	6	0	0
<i>C. perfringens</i>	10	0	0
<i>Clostridium</i> spp.	10	0	10
<i>E. lentum</i>	5	0	20
<i>Prop. acnes</i>	9	0	0
<i>Bifidobacterium</i> spp.	3	0	0

TABLE III
Correlation between penicillin resistance ($MIC \geq 16 \mu g/ml$) and production of beta-lactamase

Species	Number of resistant strains tested	Number (percent) of strains that produced		
		Penicillinase	Cephalosporinase	Both
<i>B. fragilis</i>	9	1(11)	6(67)	1(11)
<i>B. vulgatus</i>	8	0(0)	7(88)	0(0)
<i>B. distasonis</i>	4	1(25)	4(100)	1(25)
<i>B. thetaiotaomicron</i>	6	0(0)	6(100)	0(0)
<i>B. melaninogenicus</i> ss. <i>mel.</i>	5	3(60)	5(100)	3(60)
<i>B. mel.</i> ss. <i>intermedius</i>	3	1(33)	1(33)	1(33)
<i>B. asaccharolyticus</i>	6	5(83)	5(83)	5(83)
<i>F. nucleatum</i>	1	0(0)	0(0)	0(0)
<i>F. mortiferum</i>	3	0(0)	0(0)	0(0)
<i>Peptostr. anaerobius</i>	8	0(0)	0(0)	0(0)
<i>C. sphenoides</i>	1	0(0)	0(0)	0(0)
TOTAL	54	11(20)	34(63)	11(20)

DISCUSSION

The concept that penicillin has been and, for the most part, still is the most effective treatment for anaerobic infection has become firmly established among many clinicians. Reluctance by laboratories to do routine sensitivity tests of anaerobes is often based on this assumption, but the assumption is not always true. The *Bacteroides* group is the most antibiotic-resistant group of anaerobes and this resistance appears to have increased. Thadepalli, Gorbach and Bartlett (1977) reported that all *B. fragilis* isolates were inhibited by penicillin at concentrations of $\leq 128 \mu\text{g/ml}$ and Monif *et al.* (1978) reported that 88% were inhibited by $\leq 100 \mu\text{g/ml}$; however, Marrie *et al.* (1981) reported only 68% sensitive to penicillin. Moreover, Ingham *et al.* (1968) found that all *B. fragilis* strains were inhibited by tetracycline at concentrations of $\leq 0.82 \mu\text{g/ml}$ but Martin *et al.* (1972) reported only 39% of their strains sensitive to $6.2 \mu\text{g/ml}$. Increased resistance of *C. perfringens* has also been reported. Dubois, Pechere and Turgeon (1978) reported that all strains were sensitive to penicillin $0.5 \mu\text{g/ml}$ whereas Marrie *et al.* (1981) found that inhibition of all strains was achieved only at $16 \mu\text{g/ml}$. We have also found resistant isolates among the *B. melaninogenicus* group in our hospital (unpublished data). New beta-lactam antibiotics have been developed to counteract this increasing resistance and studies with some of them are reported here.

The newer beta-lactams—N-F-thienamycin and moxalactam—showed significantly more in vitro activity than the other beta-lactams tested. N-F-thienamycin was the most effective of the beta-lactam antibiotics tested against beta-lactamase producers and nonproducers; >90% of the *B. fragilis* group and all strains of other species were inhibited by $4 \mu\text{g/ml}$. Brown, Del Bene and Collins (1981) reported that *B. fragilis* ss. *fragilis* was very susceptible; studies with 100 strains gave a MIC_{90} of $0.25 \mu\text{g/ml}$. Tally, Jacobus and Gorbach (1980) also found that it inhibited anaerobic cocci and *Clostridium* spp. at 0.25 and $4 \mu\text{g/ml}$ respectively.

Our results with moxalactam were similar to those reported by Jorgensen, Crawford and Alexander (1980) and Brown *et al.* (1981) except that several strains of *Bacteroides* in our series had MICs $> 64 \mu\text{g/ml}$. Most strains of *Fusobacterium* spp. were inhibited by $8 \mu\text{g/ml}$. Our results with ampicillin and cyclacillin agree with those of Bach and Thadepalli (1980); these two antibiotics were less effective than penicillin G against *B. fragilis* and most other *Bacteroides*. We found, however, that cyclacillin was more effective than penicillin G and ampicillin against the pigmented *Bacteroides*, and against the *Fusobacterium* spp. ampicillin was slightly more effective than cyclacillin and penicillin G. Amoxycillin did not have significantly greater in vitro activity than ampicillin, cyclacillin or penicillin G.

The beta-lactamases of many aerobic and facultatively anaerobic organisms have been classified and characterised. Some of the enzymes have more than one substrate and can be controlled by either chromosomal or plasmid genes (Sykes and Matthew, 1976). Beta-lactamases of anaerobes are less defined. Our tests detected the presence in suspensions of the test strains of enzymes that would cleave the beta-lactam ring of the penicillin or cephalosporin substrate. Thus, we could detect penicillinases and cephalosporinases. Our results indicate that beta-lactamase production is confined mainly to the *Bacteroides* spp. Cephalosporinase was the most common enzyme; penicillinase was detected less frequently.

By our criterion of penicillin G resistance ($\text{MIC} \geq 16 \mu\text{g/ml}$), resistance and beta-lactamase production correlate poorly. About two thirds of the penicillin-resistant isolates produced cephalosporinase—three times more than produced penicillinase. Penicillinase and cephalosporinase activities were detected in only 20% of the resistant isolates and 80% of the penicillin-resistant strains showed no beta-lactamase activity against penicillin, which indicates that there may be other mechanisms of penicillin resistance. In penicillin-susceptible isolates penicillinase activity was detected in only one (0.8%) strain of *B. melaninogenicus* but 13% of susceptible strains produced cephalosporinase. Gill, Manning and Ingalls (1981) reported that 3% of penicillin G-sensitive ($\text{MIC} \leq 0.05$) strains of *Staphylococcus aureus* produced beta-lactamase.

Others have found that resistance to beta-lactam antibiotics among anaerobes and beta-lactamase production are not necessarily synonymous (Brown *et al.*, 1981; Gaby, Sutter and Finegold, 1981). An intrinsic resistance that may be due to a permeability barrier for the antibiotic or to crypticity of the beta-lactamase, or both, may be involved (Brown *et al.*, 1981). Alternatively, our assay may not be sufficiently sensitive to detect low levels of beta-lactamase production. Others have shown that certain organisms must be disrupted before beta-lactamase can be detected (Nord, Dornbusch and Olsson, 1980; Brown *et al.*, 1981). Additionally Weinrich and Del Bene (1976) found no specific pattern of beta-lactamase production and resistance to penicillin in *B. fragilis*.

The present study has drawn attention to the high incidence of resistance to beta-lactams in anaerobes. Two new agents, N-F-thienamycin and moxalactam, were the most active against resistant strains. Ampicillin, amoxycillin, cyclacillin and penicillin G were ineffective against most *Bacteroides* and *Fusobacterium* strains. Our results agree with reports that *Bacteroides* and *Fusobacterium* spp. have become more resistant to many of the commonly used beta-lactam antibiotics (Sutter and Finegold, 1976; Appelbaum and Chatterton, 1978; Finegold, 1979; Sanders and Lewis, 1980; Gill *et al.*, 1981). Our experiments have also shown that beta-lactamase production does not necessarily correlate with beta-lactam resistance and indicate that standardised methods for sensitivity testing of anaerobes are necessary.

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REFERENCES

- APPELBAUM, P. C., AND CHATTERTON, S. A. 1978. Susceptibility of anaerobic bacteria to ten antimicrobial agents. *Antimicrobial Agents and Chemotherapy*, **14**, 371–376.
- BACH, V. T., AND THADEPALLI, H. 1980. Susceptibility of anaerobic bacteria *in vitro* to 23 antimicrobial agents. *Chemotherapy*, **26**, 344–352.
- BAWDON, R. E., ROZMIEJ, E., PALCHAUDHURI, S. AND KRAKOWIAK, J. 1979. Variability in the susceptibility pattern of *Bacteroides fragilis* in four Detroit area hospitals. *Antimicrobial Agents and Chemotherapy*, **16**, 664–666.
- BODNER, S. J., KOENIG, M. G., TREANOR, L. L., AND GOODMAN, J. S. 1972. Antibiotic susceptibility testing of *Bacteroides*. *Antimicrobial Agents and Chemotherapy*, **2**, 57–60.
- BROWN, J. E., DEL BENE, V. E., AND COLLINS, C. D. 1981. *In vitro* activity of N-formimidoyl-thienamycin, moxalactam, and other new beta-lactam agents against *Bacteroides fragilis*: contribution of beta-lactamase to resistance. *Antimicrobial Agents and Chemotherapy*, **19**, 248–252.

- DUBOIS, J., PECHERE, J. C., AND TURGEON, P. 1978. Activity of ten antimicrobial agents against anaerobic bacteria. *Journal of Antimicrobial Chemotherapy*, **4**, 329–334.
- FINEGOLD, S. M. 1979. Anaerobic infections. *Archives of Internal Medicine*, **129**, 144–145.
- FINEGOLD, S. M., AND HEWITT, W. L. 1956. Antibiotic sensitivity pattern of *Bacteroides* species. In *Antibiotics annual*, edited by H. Welch and F. Marti-Ibanez, Medical Encyclopedia, Inc., New York, pp. 794–801.
- GABAY, E. L., SUTTER, V. C., AND FINEGOLD, S. M. 1981. Beta-lactamase testing in *Bacteroides*. American Society for Microbiology Annual Meeting, p. 12.
- GILL, V. J., MANNING, C. B., AND INGALLS, C. M. 1981. Correlation of penicillin minimum inhibitory concentration and penicillin zone edge appearance with staphylococcal beta-lactamase production. *Journal of Clinical Microbiology*, **14**, 437–440.
- GREENBERG, R. N., SCALCINI, M. C., SANDERS, C. V. AND LEWIS, A. C. 1979. Cefamandole therapy in anaerobic infections. *Antimicrobial Agents and Chemotherapy*, **15**, 337–341.
- INGHAM, H. R., SELKON, J. B., CODD, A. A., AND HALE, J. H. 1968. A study *in vitro* of the sensitivity to antibiotics of *Bacteroides fragilis*. *Journal of Clinical Pathology*, **21**, 432–436.
- JORGENSEN, J. H., CRAWFORD, S. A., AND ALEXANDER, G. A. 1980. Comparison of moxalactam (LY127935) and cefotaxime against anaerobic bacteria. *Antimicrobial Agents and Chemotherapy*, **17**, 901–904.
- MARRIE, T. J., HALDANE, E. V., SWANTEE, C. A., AND KERR, E. A. 1981. Susceptibility of anaerobic bacteria to nine antimicrobial agents and demonstration of decreased susceptibility of *Clostridium perfringens* to penicillin. *Antimicrobial Agents and Chemotherapy*, **19**, 51–55.
- MARTIN, W. J., GARDNER, M., AND WASHINGTON, J. A. 1972. *In vitro* antimicrobial susceptibility of anaerobic bacteria isolated from clinical specimens. *Antimicrobial Agents and Chemotherapy*, **1**, 148–158.
- MITCHELL, A. A. B. 1973. Incidence and isolation of *Bacteroides* species from clinical material and their sensitivity to antibiotics. *Journal of Clinical Pathology*, **26**, 738–741.
- MONIF, G. R. G., CLARK, P. R., SHUSTER, J. J., AND BAER, H. 1978. Susceptibility of the anaerobic bacteria, group D streptococci, *Enterobacteriaceae*, and *Pseudomonas* to semisynthetic penicillins: carbenicillin, piperacillin, and ticarcillin. *Antimicrobial Agents and Chemotherapy*, **14**, 643–649.
- NASTRO, L. J., AND FINEGOLD, S. M. 1972. Bactericidal activity of five antimicrobial agents against *Bacteroides fragilis*. *Journal of Infectious Diseases*, **126**, 104–107.
- NORD, C. E., DORNBUSCH, K., AND OLSSON, B. 1980. Studies on beta-lactamase in *Bacteroides fragilis*. *Infection*, **8** (Suppl. 2), 167–170.
- SANDERS, C. V., AND LEWIS, A. C. 1980. Changing trends in the antimicrobial susceptibility pattern of anaerobic bacteria. In *Anaerobic bacteria; selected topics*, edited by D.W. Lambe, R.J. Genco, and K.J. Mayberry-Carson, Plenum Press, New York, pp. 61–68.
- STANECK, J. L., AND WASHINGTON, J. A. 1974. Antimicrobial susceptibilities of anaerobic bacteria: recent clinical isolates. *Antimicrobial Agents and Chemotherapy*, **6**, 311–315.
- SUTTER, V. L., AND FINEGOLD, S. M. 1976. Susceptibility of anaerobic bacteria to 23 antimicrobial agents. *Antimicrobial Agents and Chemotherapy*, **10**, 736–752.
- SYKES, R. B., AND MATTHEW, M. 1976. The beta-lactamases of gram-negative bacteria and their role in resistance to beta-lactam antibiotics. *Journal of Antimicrobial Chemotherapy*, **2**, 115–157.
- TALLY, F. P., JACOBUS, N. V., BARTLETT, J. G., AND GORBACH, S. L. 1975. *In vitro* activity of penicillins against anaerobes. *Antimicrobial Agents and Chemotherapy*, **7**, 413–414.
- TALLY, F. P., JACOBUS, N. V., AND GORBACH, S. L. 1980. *In vitro* activity of N-Formimidoyl Thienamycin (MK0787). *Antimicrobial Agents and Chemotherapy*, **18**, 642–644.
- THADEPALLI, H., GORBACH, S. L., AND BARTLETT, J. G. 1977. Apparent failure of chloramphenicol in the treatment of anaerobic infections. *Current Therapeutic Research*, **22**, 421–426.
- WEINRICH, A. E., AND DEL BENE, V. E. 1976. Beta-lactamase activity in anaerobic bacteria. *Antimicrobial Agents and Chemotherapy*, **10**, 106–111.
- ZABRANSKY, R. J., JOHNSTON, J. A., HAUSER, K. J. 1973. Bacteriostatic and bactericidal activities of various antibiotics against *Bacteroides fragilis*. *Antimicrobial Agents and Chemotherapy*, **3**, 152–156.