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National Committee for Clinical Laboratory Standards Agar Dilution Susceptibility Testing of Anaerobic Gram-Negative Bacteria

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One hundred nine recent clinical isolates of anaerobic gram-negative bacteria were tested in triplicate by the National Committee for Clinical Laboratory Standards agar dilution procedure for their susceptibility to 32 antimicrobial agents. All isolates were inhibited by imipenem, but there were significant numbers of strains resistant to other beta-lactam drugs, and therefore the in vitro response to these antimicrobial agents cannot be predicted. This was particularly true for the bile-resistant or *Bacteroides fragilis* group. β-Lactamase production was detected in 82% of the bacteroides with the nitrocefin test. Clavulanic acid combined with amoxicillin and ticarcillin and sulbactam combined with ampicillin resulted in synergistic activity against all β-lactamase-positive organisms. Ceftizoxime was the most active of the cephalosporins. Two percent of the isolates were resistant to chloramphenicol and metronidazole. Clindamycin resistance was detected in 38% of the *B. fragilis* group, which is a marked increase from the 4% detected 10 years ago at this institution.

Susceptibility testing of anaerobic bacteria has been accepted and recommended during the last few years (4, 27, 31). This change is attributable to the development of a standardized reference method (26) and convenient-to-use broth microdilution systems. Another major factor has been the recognition during the last decade of resistance among anaerobic bacteria. Prior to 1980, a few drugs were believed to be effective against almost all anaerobic bacteria. However, resistance to most of the traditionally used antimicrobial agents and most of the newer beta-lactam antibiotics has been increasingly reported (27, 31, 37). Furthermore, Cuchural and Tally (10) have found that resistance varies with geographical area and even from time to time within the same institution.

The purpose of this study was to examine a large variety of antimicrobial agents by the reference agar dilution method. The bacteria tested were recent clinical isolates of the most commonly occurring anaerobic gram-negative bacteria. Nine years ago at this institution, the first clinical isolates of the *Bacteroides fragilis* group resistant to clindamycin were detected by the agar dilution technique (8). I was interested in comparing the results for current clinical isolates with results from the previous study and in examining newer antimicrobial agents.

MATERIALS AND METHODS

Microorganisms. One hundred nine recent clinical isolates of anaerobic gram-negative bacteria were isolated from specimens submitted to the clinical microbiology laboratory at Hutzel Hospital in the Detroit Medical Center. Strains were tested from August 1986 to March 1987. Species identification was performed by standard methods, including gas-liquid chromatography (17), bile resistance, lipase production, and the API 20A test system (Analytab Products, Inc., Plainview, N.Y.). Seventy of the strains were bile-resistant species of *Bacteroides* and were identified as *B. fragilis* (n = 24), *B. distasonis* (n = 14), *B. vulgatus* (n = 11), *B. ovatus* (n = 11), *B. thetaiotaomicron* (n = 7), *B. uniformis* (n = 1), *B. caccae* (n = 1), and *B. eggerthii* (n = 1). The *Bacteroides* species sensitive to bile consisted of 27 strains identified as *B. bivius* (n = 8), *B. disiens* (n = 2), *B. capillosus* (n = 2), *B. gracilis* (n = 3), *B. ureolyticus* (n = 5), *B. oralis* (n = 1), *B. buccae* (n = 2), *B. melaninogenicus* (n = 2), and *B. asaccharolyticus* (n = 2). The third group consisted of 12 strains identified as *Fusobacterium nucleatum* (n = 6), *F. russii* (n = 2), *F. mortiferum* (n = 1), and *Veillonella parvula* (n = 3). Strains were subcultured on Columbia anaerobic sheep blood agar (anBAP) (GIBCO Laboratories, Madison, Wis.). Fresh subcultures were prepared for each day of testing. The quality control strain *B. thetaiotaomicron* ATCC 29741 was included in all susceptibility test procedures.

Antimicrobial agents. Thirty-two antimicrobial agents were included in the study. Standard powders were obtained from the following companies: cefmenoxime, Abbott Laboratories, Chicago, Ill.; carbenicillin, amoxicillin, ticarcillin, and clavulanic acid (Augmentin and Timentin), Beecham Laboratories, Bristol, Tenn.; penicillin G, Bristol Laboratories, Syracuse, N.Y.; cefuroxime and ceftazidime, Glaxo, Inc., Durham, N.C.; cefotaxime, Hoechst-Roussel Pharmaceutical Inc., Somerville, N.J.; ceftriaxone, Hoffmann-La Roche Inc., Nutley, N.J.; piperacillin, Lederle Inc., Pearl River, N.Y.; cephalothin, erythromycin, moxalactam, and cefamandole, Lilly Research Laboratories, Indianapolis, Ind.; cefoxitin and imipenem, Merck, Sharp and Dohme, Rahway, N.J.; azlocillin, ciprofloxacin, and mezlocillin, Miles Pharmaceuticals, West Haven, Conn.; chloramphenicol, Parke-Davis/Warner Lambert, Morris Plains, N.J.; cefoperazone, ampicillin, and sulbactam (Unasyn), Pfizer Pharmaceuticals, Groton, Conn.; metronidazole, G. D. Searle and Co., Skokie, Ill.; cefonicid and ceftizoxime, Smith, Kline and French, Philadelphia, Pa.; tetracycline and aztreonam, E. R. Squibb Inc., Princeton, N.J.; cefotetan, Stuart Pharmaceuticals, Wilmington, Del.; and clindamycin, The Upjohn Co., Kalamazoo, Mich.

The antimicrobial powders were weighed, corrected for potency, and dissolved as directed by the manufacturers. Preparations at 20 times the highest concentration and serial twofold dilutions were made for the range of concentrations to be tested by the agar dilution procedure.

Media and inocula. Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.) was used in the agar dilution plates as

specified by the reference method (26). Organisms were streaked on anBAP for fresh growth. Portions of 5 to 10 colonies were suspended in enriched thioglycolate medium (BBL Microbiology Systems, Cockeysville, Md.) and incubated overnight. The broth cultures were adjusted to one-half of a number 1 McFarland turbidity standard in fresh brucella broth (BBL Microbiology Systems). The adjusted suspensions were placed into the wells of the Steers replicator block and inoculated to the surface of the individual agar dilution plates. The final inoculation density was 10^5 to 10^6 CFU per spot. For the inoculum effect studies, organisms were grown for 24 h and used undiluted and in serial 10-fold dilutions. The inoculum used for each of these dilutions was carefully checked by preparing 10-fold dilutions of each inoculum and plating 0.01-ml samples of each to anBAP. These plates were incubated for 48 h, and CFU were counted. Dilutions containing between 30 and 300 CFU were used for determining the inoculum concentration.

Susceptibility testing. The agar dilution procedure was carried out as described by the National Committee for Clinical Laboratory Standards (NCCLS) (26). Triplicate agar dilution plates were made on the day the drugs were prepared and were used within 1 week, with the exception of imipenem, carbenicillin-clavulanic acid, amoxacillin-clavulanic acid, ampicillin-sulbactam, and aztreonam. These five drugs were prepared on the three separate days they were used. Seven serial twofold dilutions were tested for each drug except ticarcillin-clavulanic acid, amoxicillin-clavulanic acid, metronidazole, ampicillin, ampicillin-sulbactam, and cefoxitin which had, respectively, 11, 10, 10, 9, 9, and 9 dilutions each. The agar surfaces were inoculated with the adjusted organism suspension and incubated in an anaerobic chamber (Coy Manufacturer, Ann Arbor, Mich.) with an atmosphere of 85% nitrogen, 5% carbon dioxide, and 10% hydrogen at 35°C for 48 h. *B. thetaiotaomicron* ATCC 29741 was included on all plates. All results were within the NCCLS-recommended range. All clinical strains had endpoints within one dilution, but when two different endpoints were obtained for the triplicate testing, the value obtained in at least two of the three readings was used.

β-Lactamase test. All strains were tested by using growth from the anBAP. Nitrocefin-containing disks were used as specified by the manufacturer (BBL Microbiology Systems).

RESULTS

Eleven isolates initially selected for use in this study would not grow on the growth control Wilkins-Chalgren medium and were not included in the study. These organisms were *Bacteroides disiens* (n = 2), *B. bivius* (n = 4), *B. capillosus* (n = 2), *B. oralis* (n = 1), *B. melaninogenicus* (n = 1), and *Fusobacterium nucleatum* (n = 1).

One hundred nine recent clinical isolates were included in this study. The *Fusobacterium* and *Veillonella* isolates were susceptible to all antimicrobial agents tested. The range of inhibitory concentrations of each antimicrobial agent and the MICs required to inhibit 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively) for the *Bacteroides* species are shown in Table 1. For the bile-resistant *Bacteroides* species, MIC_{50}s and MIC_{90}s were higher than for the bile-sensitive organisms when tested against most beta-lactam drugs. However, imipenem was active against all groups, with only two isolates of *B. distasonis*, one of *B. caccae*, and one of *B. disiens* having MIC endpoints of 2 μg/ml. MICs for all other isolates were <1 μg/ml. The combinations of antibiotics and β-lactamase inhibitors were effective against all anaerobic bacteria tested. The effect of the inhibitors was most dramatic with the *Bacteroides* species (Table 1). The MIC_{50} and MIC_{90} of ampicillin for the bile-resistant group were 32 and >128 μg/ml, respectively. However, the ampicillin-sulbactam combination had corresponding MIC_{50}s and MIC_{90}s of 1 and 8 μg/ml, respectively. A similar decrease was observed for the bile-sensitive group and was also seen with amoxicillin, amoxicillin-clavulanic acid, ticarcillin, and ticarcillin-clavulanic acid (Table 1).

All isolates were tested for production of β-lactamase by using the nitrocefin-containing disks. The bile-resistant *Bacteroides* group isolates were 91% positive for β-lactamase production, and the bile-sensitive group isolates were 63% positive. No β-lactamase activity was detected among the *Fusobacterium* or *Veillonella* isolates.

Cefoxitin had an MIC_{50} and MIC_{90} of 16 and 64 μg/ml and cefotetan had an MIC_{50} and MIC_{90} of 32 and >32 μg/ml, respectively, for the bile-resistant group. The broad-spectrum cephalosporin with the lowest values was ceftizoxime, with an MIC_{50} and MIC_{90} of ≤4 and 32 μg/ml for bile-resistant organisms and ≤4 and 16 μg/ml for bile-sensitive organisms, respectively.

The effect of differences in inoculum size was examined for cefoxitin, cefotetan, and ceftizoxime with four clinical isolates and the control strain *B. thetaiotaomicron* ATCC 29741. The agar dilution reference method specifies 10^5 to 10^6 CFU per spot on the agar surface. This requires an inoculum of 10^8 CFU/ml. Cefoxitin and cefotetan were not affected by inoculum size ranging from 10^4 to 10^8 CFU per spot, with all endpoints falling within two dilutions. However, ceftizoxime demonstrated a direct effect with significantly higher MICs when the concentrations of CFU were increased. This was particularly evident with a *B. thetaiotaomicron* clinical isolate for which the MIC of ceftizoxime was 8 μg/ml at an inoculum of 10^4 or 10^5 CFU per spot, 16 μg/ml at 10^6 CFU, 64 μg/ml at 10^7 CFU, and 128 μg/ml at 10^8 CFU. For the ATCC strain the MIC was 4 μg/ml for inocula from 10^4 to 10^7 but 64 μg/ml at 10^8 CFU per spot. Less dramatic but direct correlations were observed for increasing MICs with increasing inocula for the *B. fragilis*, *B. distasonis*, and *B. ovatus* strains.

Resistance to clindamycin continues to occur in this institution among *Bacteroides* species. Sixty-two percent of the bile-resistant strains were inhibited by ≤4 μg of clindamycin per ml. The MIC_{50} and MIC_{90} for *Bacteroides* species were as follows: *B. fragilis*, <0.25 and >16 μg/ml; *B. distasonis*, 2 and >16 μg/ml; *B. vulgatus*, >16 and >16 μg/ml; *B. ovatus*, 2 and >16 μg/ml; *B. thetaiotaomicron*, 4 and >16 μg/ml; *B. caccae* (1 strain), >16 μg/ml; *B. gracilis*, < 0.25 and >16 μg/ml; and *B. melaninogenicus*, ≤0.25 and >16 μg/ml. All other organisms were inhibited by ≤4 μg of clindamycin per ml. The MIC of metronidazole was always ≤2 μg/ml, except for one strain of *B. bivius*, for which the MIC was 16 μg/ml. This organism was rechecked for identification and susceptibility testing, and the results were confirmed. Only two isolates were resistant to chloramphenicol (MIC, ≥16 μg/ml) and for 98% of the isolates MICs were ≤8 μg/ml.

DISCUSSION

Clindamycin-resistant strains of the *B. fragilis* group were first observed in 1978 by using Wilkins-Chalgren medium in an agar dilution susceptibility test (8). This observation led to a survey of Detroit hospitals for the incidence of clindamycin resistance among *B. fragilis* isolates (5). The general

TABLE 1. Susceptibility of bile-resistant and bile-sensitive *Bacteroides* species to 32 antimicrobial agents

Test agent	MIC (μg/ml) for *Bacteroides* species[a]					
	Bile resistant			Bile sensitive		
	Range	50%	90%	Range	50%	90%
Penicillin	0.25–>16	16	>16	≤0.12–>16	4	16
Ampicillin	≤0.5–>128	32	>128	≤0.5–64	2	16
Ampicillin-sulbactam (2:1 ratio)	≤0.5–16	1	8	<0.5–4	<0.5	2
Amoxicillin	≤2–>128	32	>128	≤2–128	<2	64
Amoxicillin-clavulanic acid (2:1 ratio)	<0.25–8	0.5	4	<0.25–2	0.5	1
Carbenicillin	≤8–>512	32	512	≤8–32	<8	32
Ticarcillin	≤4–>256	32	>256	≤4–32	<4	16
Ticarcillin-clavulanic acid (2 μg/ml)	<0.25–32	1	16	<0.25–32	<0.25	8
Azlocillin	≤4–>256	32	>256	≤4–64	8	32
Mezlocillin	≤4–>256	16	256	≤4–256	≤4	32
Piperacillin	≤4–>256	8	256	≤4–64	8	32
Aztreonam	8–>64	>64	>64	≤1–>64	4	>64
Cephalothin	≤0.5–>32	>32	>32	≤0.5–>32	4	>32
Cefoxitin	≤0.5–128	16	>64	≤0.5–8	2	8
Cefamandole	≤2–>128	64	>128	≤2–64	≤2	32
Cefotetan	≤0.5–>32	32	>32	≤0.5–16	2	16
Cefonicid	≤1–>64	>64	>64	≤1–>64	4	64
Cefuroxime	≤1–>64	64	>64	≤1–>64	4	64
Cefotaxime	≤0.5–>32	32	>32	≤0.5–>32	2	16
Cefmenoxime	≤2–>128	16	128	≤2–>128	<2	16
Ceftizoxime	≤4–128	≤4	32	≤4–128	<4	16
Ceftriaxone	≤2–>128	32	128	≤2–>128	<2	32
Ceftazidime	≤1–>64	>64	>64	≤1–>64	4	32
Cefoperazone	≤2–>128	32	>128	≤2–64	4	16
Moxalactam	≤2–>128	8	128	≤2–32	2	16
Imipenem	≤0.5–16	≤0.5	≤0.5	≤0.5–2	≤0.5	≤0.5
Clindamycin	≤0.25–>16	2	>16	≤0.25–>16	<0.25	>16
Erythromycin	≤0.5–>32	16	32	≤0.5–>32	2	>32
Tetracycline	≤0.5–>32	16	32	≤0.5–>32	8	32
Chloramphenicol	2–16	4	8	≤0.5–16	2	4
Metronidazole	≤0.12–0.5	0.25	0.5	≤0.5–16	0.5	0.5
Ciprofloxacin	≤0.5–>32	4	16	≤0.5–>32	2	8

[a] See text for individual species in each group.

occurrence and changing susceptibility patterns of *Bacteroides* species have since been reported by several investigators (1, 4, 9, 16, 20, 29, 32, 34, 36, 37). However, it is frequently difficult to evaluate some reports because of variations in technique, media, size and age of inocula, incubation conditions, and breakpoints used to determine resistance. Furthermore, if the correct MIC is near the breakpoint, procedural variations can cause major errors in interpretation. The purpose of this study was to test approximately 100 recent clinical isolates in triplicate by a standardized technique. The NCCLS procedure (26) was selected so that results could be evaluated and compared with those from a previous report from this laboratory and other investigators who select the same procedure. A drawback of this technique is the failure of a few strains to grow on Wilkins-Chalgren medium (28, 36). Ten percent of the isolates tested would not grow on this medium; however, all of the bile-resistant strains grew.

Resistance of most anaerobic gram-negative bacteria to beta-lactam antimicrobial agents is primarily mediated by the production of β-lactamases. A great variation in specific activity has been demonstrated among enzymes from different *Bacteroides* strains (14, 24, 25, 33). Penicillin, ampicillin, and amoxicillin are comparable and not very effective for inhibiting *Bacteroides* species, an observation which correlates with the high incidence of β-lactamase production among *Bacteroides* species (7, 14, 21, 22, 25, 36). Among the bile-resistant *Bacteroides* species, *B. distasonis* had the lowest percentage of isolates with demonstrable β-lactamase production. My finding of 64% is in agreement with the 58% reported by Wexler and Finegold (36). Like these investigators, I had strains of *B. distasonis* from which I was not able to demonstrate β-lactamase production, yet for all 14 *B. distasonis* isolates in this study penicillin MICs were ≥16 μg/ml. Either the nitrocefin test used for β-lactamase was not sufficiently sensitive or other mechanisms of resistance, such as binding or permeability, are at play (25). Therefore, a negative nitrocefin disk β-lactamase test should not be used as an indication of susceptibility to all β-lactamase-sensitive drugs. This is particularly true for *B. distasonis*. However, a positive β-lactamase test does correlate with resistance to the β-lactamase-sensitive drugs. Overall detection of β-lactamase production by all species of *Bacteroides* was 82%. The 87% positive result for *B. bivius* is noteworthy, although 76% of isolates of this species have previously been reported positive (21). All *Fusobacterium* and *Veillonella* species tested were β-lactamase negative and susceptible to penicillin, which agrees with a recent report (28).

One approach to overcoming the resistance to beta-lactam drugs has been the development of β-lactamase inhibitors. Fekete et al. (15) demonstrated a marked reduction in MIC_{50}s and MIC_{90}s with two inhibitors, clavulanic acid and sulbactam, when they were combined with cefazolin and tested against *B. fragilis*. I observed synergistic activity for amoxicillin-clavulanic acid compared with amoxicillin alone when tested against all the *Bacteroides* isolates which were

β-lactamase positive. In contrast, the combination has no effect on reducing the MICs for the five β-lactamase-negative strains. The nitrocefin disk test did predict in vitro synergistic activity of the β-lactamase inhibitors. These results are in agreement with other reports (1, 22). The same activity was observed for ticarcillin and ticarcillin-clavulanic acid, as previously reported for combinations of clavulanic acid with beta-lactam compounds (2, 3, 7, 11, 15, 22). Sulbactam has also been reported to augment the activity of beta-lactam drugs when tested against *Bacteroides* species (2, 15). I observed the synergy for ampicillin combined with sulbactam. The range of MICs of ampicillin was 16 to >128 μg/ml for *B. fragilis*, but the MIC dropped to ≤0.5 to 4 μg/ml when ampicillin was combined with sulbactam in a 2:1 ratio. A similar decrease in MIC endpoints was observed for all *Bacteroides* species, with ampicillin MICs of ≥8 μg/ml. This synergistic reaction occurred among the bile-resistant and bile-sensitive groups. Again, there was a direct correlation with a positive nitrocefin test and synergy. Most members of the genus *Bacteroides* are susceptible in vitro to amoxicillin, ampicillin, and ticarillin when the β-lactamases are inhibited.

Another approach to overcoming bacterial resistance has been the development of drugs which are more resistant to degradation by β-lactamase enzymes. Cephalothin and other early cephalosporin antibiotics have been found to be ineffective against bacteroides (13). Some of the molecular modifications include the 7α-methoxy cephalosporins like cefoxitin, moxalactam, and cefotetan and the 7β-methoxyiminoacetamide cephalosporin group represented by cefotaxime and cefmenoxime. These and related drugs have been evaluated for in vitro activity against *Bacteroides* species, particularly the bile-resistant group (1, 6, 9, 13, 20, 29, 34). Each of these studies examined a few antibiotics, but none tested the large number of drugs included in this report. My results are in agreement with most of the reports. However, the ceftizoxime MIC_{50} and MIC_{90} of ≤4 and 32 μg/ml, respectively, for the *B. fragilis* group were considerably lower than the 32 and >128 μg/ml reported by File et al. (16) but in agreement with the 4 and 16 μg/ml reported by Thornsberry (34) and the 2 and 16 μg/ml of Aldridge et al. (1). The percent susceptible at a ≤16-μg/ml breakpoint for the individual species of the bile-resistant group from this study and the reports of Aldridge et al. (1) and File et al. (16) are as follows: *B. fragilis*, 79, 95, and 37%; *B. distasonis*, 86, 92, and 29%; *B. vulgatus*, 64, 100, and 35%; and *B. thetaiotaomicron*, 86, 62, and 24%, respectively. The difference could be due to different strains from various geographical areas (32) or even from different hospitals in the same area (5, 16). It is noteworthy that most of the resistant strains in the report of File et al. (16) came from smaller community hospitals and not the large institution in their study.

Eley and Greenwood (13) reported a marked inoculum effect for ceftizoxime between 10^3 and 10^5 CFU per inoculum. We tested organisms at 10-fold dilutions from 10^8 to 10^4 CFU per inoculum spot. A significant increase was observed in the MIC of ceftizoxime as the inoculum size increased. My testing differed in that I carried the inoculum to higher concentrations, used different media to grow the organisms and for the agar dilution testing, and did colony counts to confirm the inocula. However, I also observed the inoculum effect reported by Eley and Greenwood and demonstrated that it continued for certain strains to even higher values with corresponding increases in inocula. This is in agreement with Borobio et al. (6), who demonstrated that MIC_{90}s increased with increasing inocula but the organisms remained susceptible (≤32 μg/ml) at 10^3 to 10^7 CFU per inoculum.

Cefoxitin is frequently used for treating anaerobic infections. I observed MICs greater than the breakpoint (susceptible if ≤16 μg/ml) for 49% of the *B. fragilis* group but none of the bile-sensitive strains and none of the *Fusobacterium* or *Veillonella* strains. The proportion of the bile-resistant group consisting of the more resistant species (*B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron*) (45%) is similar to that of other studies (1, 37) and does not explain the increased resistance to cefoxitin. Selection of and agreement on breakpoints for susceptibility testing have been a source of disagreement and confusion. I applied NCCLS values, which recommend an MIC of ≤16 μg/ml as indicating susceptible or moderately susceptible. When this cutoff was used in the study of Sutter et al. (30), they found 40% of strains were resistant to cefoxitin among their 1979 isolates of the *B. fragilis* group rather than the 8% at 32 μg/ml. *B. fragilis* is more susceptible to the 7α-methoxy cephalosporins than other species in the bile-resistant group. The MICs of cefotetan were slightly lower than those of cefoxitin for *B. fragilis*, but other bile-resistant strains were more resistant to cefotetan than cefoxitin. Cefonicid was not effective against *Bacteroides* species.

The broad-spectrum penicillins and relatively penicillinase-resistant drugs azlocillin and mezlocilin were not very effective against the bile-resistant strains. The MIC_{50} and MIC_{90} of azlocillin were 32 and >256 μg/ml, respectively, for the *B. fragilis* group, compared with 8 and 32 μg/ml in an earlier report (1). The corresponding values for mezlocillin were 16 and 256 μg/ml versus the 8 and 32 μg/ml noted previously. These differences may again be explained by geographical differences or the 4-year difference in time of collection of isolates in the two studies.

Imipenem was the most effective beta-lactam drug in this study. All organisms were inhibited by ≤2.0 μg/ml, and this drug may have a role in treating anaerobic infections if there is resistance to other drugs. However, it is not a universal antianaerobe drug, since occasional resistant strains of *B. fragilis* have been reported (23).

The monobactam aztreonam had very little activity against the anaerobic gram-negative bacteria, which is in agreement with the report that *Bacteroides* spp. are intrinsically resistant to monobactams (18). However, Jacobus et al. did report synergy for aztreonam and clavulanic acid for 5 of 15 strains of *B. fragilis*.

Overall, the results of this study agree with those of Talley et al. (32) in their nationwide survey. However, this investigation revealed an increase of at least two $\log_2$ dilutions in the MIC_{50} and MIC_{90} of moxalactam and clindamycin and the MIC_{90} of piperacillin. In contrast, there was a two-$\log_2$ decrease in the MIC_{50} and MIC_{90} of metronidazole in this report. Two major differences between these two studies were the use of brain heart infusion broth and agar by Tally et al. (32) and Wilkins-Chalgren agar in this study and the time that organisms were collected. Isolates for this study were collected during the second half of 1986 and the first 3 months of 1987, while Tally et al. tested bacteria collected in 1983.

The clindamycin results are of special interest since MICs were ≥8 μg/ml for only 4% of 231 *B. fragilis* group isolates collected between September 1977 and August 1979 but for 38% of 70 isolates in this study. The methods used and the hospital were the same in both studies. The MICs for only 5 of 169 (3%) bile-sensitive bacteroides were ≥8 μg/ml in the previous report, but 3 of 27 (11%) exceeded the breakpoint in this study. The three organisms were *B. gracilis*, *B. disiens*, and *B. capillosus*. The clinical importance of *B. gracilis* has been noted recently (19).

Tetracycline continues to be ineffective for *Bacteroides* species, with the MIC for the majority of strains being ≥4 μg/ml. In contrast, chloramphenicol continues to be very effective, as demonstrated by only 2% resistant strains (MIC, ≥16 μg/ml) in the previous study (8) and again only 2% resistant in this report, one isolate of *B. distasonis* and one of *B. disiens*.

The quinolone ciprofloxacin was tested for its activity. The MIC_{50} data demonstrated that the amount of ciprofloxacin required to inhibit the *Bacteroides* spp. exceeded the peak levels in serum (approximately 2 μg/ml). The majority of the anaerobic gram-negative bacteria were resistant to ciprofloxacin, which agrees with other recent reports (12, 35).

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