JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1986, p. 545–550
0095-1137/86/030545-06$02.00/0

Vol. 23, No. 3

Evaluation of Broth Disk Elution Methods for Susceptibility Testing of Anaerobic Bacteria with the Newer β-Lactam Antibiotics

J. H. JORGENSEN,* J. S. REDDING, AND A. W. HOWELL

Department of Pathology, The University of Texas Health Science Center, San Antonio, Texas 78284

Received 23 August 1985/Accepted 3 December 1985

Broth disk elution procedures represent one of the most practical means for clinical laboratories to perform routine antibiotic susceptibility tests on anaerobic bacteria. The accuracy of five disk elution test methods and media (including the one to be proposed by the National Committee for Clinical Laboratory Standards) was evaluated for the testing of newer beta-lactam antibiotics, including cefoperazone, cefotaxime, cefoxitin, ceftazidime, ceftizoxime, moxalactam, and piperacillin. Various numbers of antibiotic disks were used to achieve disk elution test concentrations which approximated the highest MIC termed susceptible by the Food and Drug Administration. A group of 88 anaerobes representing many different species was tested in parallel by the five disk elution methods and the National Committee for Clinical Laboratory Standards reference agar dilution procedure. Overall, full agreement between the reference agar dilution MICs and the disk elution category results was 88.3% for PRAS BHI, 84.5% for Schaedler, 85.7% for thioglycolate, and 87.4% for Wilkins-Chalgren broth. Essential agreement (±1 twofold MIC increment from the disk elution concentration) was achieved with 94.6% of PRAS BHI tests, 94.3% of Schaedler tests, 93.6% of thioglycolate tests, and 95.7% of Wilkins-Chalgren tests. Due to growth failures with a number of isolates and difficulties in interpreting results, the use of Wilkins-West broth was discontinued after approximately one-half of the isolates had been tested. The majority of errors with all of the disk elution methods occurred with isolates (most notably members of the *Bacteroides fragilis* group) having MICs near the single test concentrations used in the disk methods. With the notable exception of tests for the *B. fragilis* group, the disk elution methods offered acceptable accuracy with the newer beta-lactam antibiotics tested in this study.

The importance of anaerobic bacteria in a number of infectious processes has become widely recognized during the past 2 decades. During this period, the potential for antimicrobial agent resistance has risen to the point that anaerobes are no longer entirely predictable in their susceptibility to commonly used drugs (2, 7, 28, 31, 34). Recently, several beta-lactam antibiotics have been introduced which have variable activities against anaerobes (22, 25). Thus, for selection of optimal therapy, it may now be necessary for clinical microbiology laboratories to determine routinely the susceptibility of anaerobes on each isolation (9, 26). While the agar dilution test method described by the National Committee for Clinical Laboratory Standards (NCCLS; 21) appears to be the most reproducible method currently available, most clinical laboratories will not find it convenient for routine use. Likewise, broth microdilution tests (12, 17, 23, 27) may not be suitable for use in all laboratories. The broth disk elution methods (13, 15, 37, 39) appear to offer practicality and accuracy for routine testing of anaerobes with older antimicrobial agents such as penicillin, chloramphenicol, and metronidazole. The purpose of this study was to carefully evaluate the use of these techniques with several newer beta-lactam antibiotics.

MATERIALS AND METHODS

Anaerobe isolates. A group of 88 anaerobe isolates of clinical origin representing many different species was employed in this study. These isolates included *Actinomyces naeslundii*, 1; *Bifidobacterium eriksonii*, 1; *Bacteroides asaccharolyticus*, 2; *Bacteroides bivius*, 5; *Bacteroides capillosus*, 1; *Bacteroides distasonis*, 9; *Bacteroides fragilis*, 20; *Bacteroides melaninogenicus*, 1; *Bacteroides ovatus*, 8; *Bacteroides thetaiotaomicron*, 10; *Bacteroides vulgatus*, 7; *Clostridium butyricum*, 1; *Clostridium cadaveris*, 1; *Clostridium difficile*, 1; *Clostridium paraputrificum*, 1; *Clostridium perfringens*, 5; *Clostridium sordellii*, 2; *Eubacterium limosum*, 1; *Fusobacterium mortiferum*, 1; *Fusobacterium necrophorum*, 1; *Peptostreptococcus asaccharolyticus*, 2; *Peptostreptococcus magnus*, 1; *Peptostreptococcus anaerobius*, 4; *Propionibacterium acnes*, 1; and *Streptococcus intermedius*, 1. In addition, *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium perfringens* ATCC 13124 were used as control organisms with each set of agar dilution MICs and were tested by each disk elution method once. Control values for agar dilution MICs either were those indicated in the NCCLS M11-A document (21) or were obtained from the respective drug manufacturers.

Reference agar dilution susceptibility tests. The NCCLS reference anaerobe agar dilution method (21) was used to evaluate the relative accuracy of the disk elution methods. This included use of Wilkins-Chalgren agar containing twofold incremental concentrations of each drug, inoculation with a Steers replicator, and incubation at 35 to 37°C for 48 h in an anaerobe chamber (85% N_2–10% H_2–5% CO_2 atmosphere). The MIC for each drug-organism combination was termed the least concentration of the drug which prevented growth, allowed growth of only one colony, or allowed only a faint haze on the agar surface.

Broth disk elution methods. (i) PRAS BHI method. The method of Wilkins and Thiel (39) was performed as follows. The test medium was PRAS BHI broth (Scott Laboratories, Inc.); the inoculum for each tube consisted of 1 drop of an overnight broth culture transferred under anaerobic conditions; tubes were incubated for 24 h at 37°C. The isolate was considered susceptible if the turbidity was <50% of that of

* Corresponding author.

TABLE 1. Overall agreement between the reference and disk elution results from tests of 88 anaerobe isolates

Disk elution test medium	No. of tests	% Full agreement[a]	% Essential agreement[a]
PRAS BHI	616	88.3	94.6
Schaedler	560	84.5	94.3
Thioglycolate	595	85.7	93.6
Wilkins-Chalgren	588	87.4	95.7
Wilkins-West[b]	287	90.9	96.5

[a] See the text for an explanation.
[b] Results from only 52 isolates (see the text).

the simultaneous control tube and resistant if turbidity was ≥50% of that of the growth control.

(ii) Schaedler broth method. The method described by Jorgensen et al. (13) was performed as follows. The test medium was Schaedler broth (with vitamin K_1; BBL Microbiology Systems); the inoculum was 1 drop of an overnight broth culture; incubation was for 24 h in a GasPak jar at 35°C. The isolate was considered susceptible if the medium remained clear or if there was a barely visible haze; otherwise, the isolate was considered resistant.

(iii) Aerobically incubated thioglycolate method. The method described by Kurzynski et al. (15) was performed as follows. The test medium was enriched thioglycolate broth (with hemin and vitamin K_1; BBL); the inoculum was 2 drops of an overnight broth culture added after 2 h of prediffusion of disks in the broth; incubation was for 24 h at 37°C in a standard (nonanaerobic) incubator. The isolate was considered susceptible if turbidity was <50% of that of the simultaneous control tube and resistant if turbidity was ≥50% of that of the growth control.

(iv) Wilkins-Chalgren broth method. The following previously unpublished method utilized Wilkins-Chalgren broth (38). The medium was prepared in our laboratory using Anaerobe Broth, Experimental dehydrate (Difco Laboratories); the inoculum was 1 drop of an overnight broth culture; incubation was for 24 h in a GasPak jar at 35°C. The isolate was considered susceptible if the medium remained clear or if there was a barely visible haze; otherwise, it was considered resistant.

(v) Wilkins-West broth method. The method described by West and Wilkins (37) was performed with 10-ml tubes of Wilkins-West broth prepared from the separate ingredients by the authors' description. The inoculum for each tube was 0.1 ml of an overnight broth culture. Tubes were sealed with Vaspar (37). The tubes were incubated for 24 h at 37°C in a standard (nonanaerobic) incubator. The isolate was considered susceptible if turbidity was <50% of that of the control tube, and resistant if turbidity was ≥50% of that of the growth control.

Antibiotics and disk elution test concentrations. The beta-lactam antibiotics included for evaluation of the various disk elution test methods were cefoperazone, cefotaxime, cefoxitin, ceftazidime, ceftizoxime, moxalactam, and piperacillin. With the exception of the method of West and Wilkins (37), which required 10 ml of broth, the same volume of broth (5 ml) was required with each of the disk elution test methods. The appropriate number of individual disks was chosen to achieve a single test concentration close to the highest MIC termed susceptible for each drug by Food and Drug Administration criteria. The MICs (in micrograms per milliliter) termed susceptible were thus; ≤16 for cefoperazone, cefotaxime, cefoxitin, ceftazidime, ceftizoxime, and moxalactam, and ≤64 for piperacillin. The actual numbers of disks employed and the final test concentrations for all of the methods except that of West and Wilkins (37) were as follows: cefoperazone, one 75-μg disk for 15 μg/ml; cefotaxime, cefoxitin, ceftazidime, ceftizoxime, and moxalactam (each), three 30-μg disks for 18 μg/ml; and piperacillin, three 100-μg disks for 60 μg/ml. Twice the number of disks was required for the West and Wilkins method to obtain the same drug concentrations, since the broth volume was 10 ml (37).

Inoculum adjustment study. A group of 10 *B. fragilis* group isolates was tested in parallel with the inoculum preparations described above for the various disk elution methods and with an adjusted inoculum prepared by the method of Swenson and Thornsberry (33). For the adjusted inoculum, colonies from a 48-h-old blood agar plate were suspended in broth to the density of the 0.5 McFarland standard. A 100-μl sample of the adjusted suspension was then used to inoculate the various test media. For this experiment, only cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and moxalactam were tested.

Disk elution tests with additional disks. In addition to testing 10 *B. fragilis* group isolates with the original and adjusted inocula as described above, tests were performed with both inocula with the standard number and twice the number of disks described above. Thus, cefoperazone was tested at 15 and 30 μg/ml, and cefotaxime, ceftazidime, ceftizoxime, and moxalactam were each tested at 18 and 36 μg/ml.

Determination of agreement between the reference and test systems. The disk elution test result was said to be in full agreement with the reference test result if the reference MIC

TABLE 2. Categorization of disk elution errors by test methods and by drugs (number of strains tested)[a]

Drug	BHI (88)		Schaedler (80)		Thioglycolate (85)		Wilkins-Chalgren (84)		Wilkins-West (41)	
	% False S	% False R	% False S	% False R	% False S	% False R	% False S	% False R	% False S	% False R
Cefoperazone	4.5, 3.4	0, 0	10.0, 8.8	0, 0	5.9, 3.5	1.2, 0	2.4, 2.4	4.8, 0	0, 4.9	4.9, 0
Cefotaxime	2.0, 0	10.2, 1.1	7.5, 3.8	6.2, 0	1.2, 1.2	8.2, 3.5	1.2, 1.2	9.5, 3.6	0, 4.9	7.3, 7.3
Cefoxitin	1.1, 0	2.3, 8.0	3.8, 0	0, 0	1.2, 0	2.4, 5.9	2.4, 0	0, 0	4.9, 0	0, 0
Ceftazidime	8.0, 0	2.3, 0	23.8, 7.5	0, 0	21.2, 7.1	0, 0	11.9, 3.6	3.6, 1.2	7.3, 2.5	0, 0
Ceftizoxime	0, 3.4	5.7, 8.0	2.5, 6.2	3.8, 7.5	0, 1.2	10.6, 11.8	2.4, 2.4	11.9, 9.5	0, 4.9	7.3, 0
Moxalactam	4.5, 1.1	3.4, 4.5	6.2, 1.2	2.5, 5.0	1.2, 1.2	2.4, 8.2	6.0, 1.2	2.4, 4.8	2.4, 0	2.4, 0
Piperacillin	0, 0	0, 8.0	1.2, 0	0, 0	0, 0	0, 1.2	0, 0	0, 0	2.4, 0	0, 0
Total error	2.9, 1.1	3.4, 4.2	7.8, 3.9	1.8, 1.8	4.4, 2.0	3.5, 4.4	3.7, 1.5	4.6, 2.7	2.4, 2.4	3.1, 1.0

[a] S, Susceptibility; R, resistance. Percentages are for 1 dilution and >1 dilution.

TABLE 3. Overall agreement between the reference and disk elution results from 378 tests of 54 *B. fragilis* group isolates

Disk elution test medium	% Full agreement[a]	% Essential agreement[a]
PRAS BHI	82.8	92.3
Schaedler	79.1	92.6
Thioglycolate	79.4	91.3
Wilkins-Chalgren	82.5	94.7

[a] See the text for an explanation.

TABLE 5. Disk elution test results for species of the *B. fragilis* group

Species	No. of isolates	No. of tests	% Full agreement[a]	% Essential agreement[a]
B. distasonis	9	252	76.2	90.5
B. fragilis	20	560	80.9	94.1
B. ovatus	8	224	82.6	91.5
B. thetaiotaomicron	10	280	88.5	94.6
B. vulgatus	7	196	74.5	90.3

[a] See the text for an explanation.

was not more than the highest susceptibility MIC (see above) and the disk elution result was "susceptible" or if the reference MIC was greater than the susceptibility MIC listed above and the disk elution result was "resistant." The disk elution test result was said to provide essential agreement if the reference MIC was not more than 1 concentration increment higher than the concentration used for the disk test and the disk elution result was "susceptible" or if the reference MIC was greater than or equal to the susceptibility MIC and the disk elution result was "resistant."

RESULTS

The disk elution test methods examined in this study varied in their ease of inoculation, result interpretation, and, to some degree, reliability of obtaining growth of the more fastidious anaerobes. PRAS BHI was the most reliable medium for promoting growth, whereas Wilkins-West broth had the largest number of growth failures (11 of 52 isolates tested). The methods with Schaedler, thioglycolate, and Wilkins-Chalgren broths were the simplest to perform, whereas the PRAS BHI and Wilkins-West broths were cumbersome due to the tedious inoculation procedures of both methods. Moreover, the growth endpoints with Wilkins-West broth were very difficult to interpret due to the settling of growth to the bottom of the tubes and the formation of a thick coagulum. Because of the additional difficulties associated with the use of Wilkins-West broth, it was deleted from further study after approximately 50% of the isolates had been tested.

All of the disk elution methods offered full agreement with the reference method for ≥84.5% of isolates and essential agreement with ≥93.6% of isolates (Table 1). At first glance, the Wilkins-West broth method appears to offer the greatest accuracy (Table 1); however, only 17 of 54 *B. fragilis* group isolates, which proved problematic by all methods, were tested in the initial group of anaerobes. After the first 52 isolates were tested, the Wilkins-West broth method appeared to offer accuracy similar to but not better than that of the other disk elution methods.

Table 2 details the errors encountered with each disk elution test method and each drug for the entire group of anaerobe isolates. The Schaedler broth method yielded slightly more false-susceptible results than the other methods, but fewer false-resistant results were observed. All of the disk elution test methods appeared to offer the greatest accuracy when cefoxitin and piperacillin were tested and lower accuracy when cefoperazone, cefotaxime, ceftizoxime, ceftazidime, and moxalactam were tested.

Many of the disk elution test errors appeared to emanate from tests involving members of the *B. fragilis* group (summarized in Table 3). Table 4 details the errors encountered among the 54 *B. fragilis* group isolates. The error rates for the various methods were more than four times the error rate encountered with all of the remaining species tested (data not shown). Still, fewer than 10% overall errors occurred with piperacillin and cefoxitin, while error rates as high as 32% were noted with the remaining drugs. False-susceptible results appeared more often with cefoperazone and ceftazidime, while false resistance was more common with ceftizoxime (Tables 2 and 4). When disk elution error rates for individual species within the *B. fragilis* group were examined, slightly fewer errors were noted with the *B. thetaiotaomicron* isolates (Table 5).

The larger number of errors seen with the *B. fragilis* group in this study were presumed to be due either to the proximity of the MICs of many strains to the test concentrations used with the newer beta-lactam antibiotics or to the rapid growth rate of these species as a possible cause of test overinoculation with the standard inocula described for the disk elution

TABLE 4. Categorization of disk elution errors encountered with *B. fragilis* group by test methods and by drugs (number of strains tested)[a]

Drug	BHI (54)		Schaedler (54)		Thioglycolate (54)		Wilkins-Chalgren (54)		Total error (%)[b]
	% False S	% False R	% False S	% False R	% False S	% False R	% False S	% False R	
Cefoperazone	7.4, 5.6	0, 0	14.8, 13.0	0, 0	9.2, 5.6	1.8, 0	3.7, 1.8	7.4, 0	17.6
Cefotaxime	3.7, 0	16.7, 1.8	11.1, 3.7	9.2, 0	1.8, 0	13.0, 3.7	1.8, 0	14.8, 5.6	21.8
Cefoxitin	1.8, 0	3.7, 11.1	5.6, 0	0, 0	1.8, 0	3.7, 7.4	3.7, 0	0, 0	9.7
Ceftazidime	11.1, 0	1.8, 0	31.5, 7.4	0, 0	29.6, 9.2	0, 0	16.7, 3.7	5.6, 0	29.1
Ceftizoxime	0, 5.6	9.2, 13.0	3.7, 7.4	5.6, 11.1	0, 0	16.7, 18.5	1.8, 1.8	18.5, 14.8	32.0
Moxalactam	5.6, 1.8	5.6, 7.4	7.4, 1.8	3.7, 7.4	1.8, 1.8	3.7, 13.0	7.4, 1.8	3.7, 7.4	20.4
Piperacillin	0, 0	0, 7.4	1.8, 0	0, 0	0, 0	0, 1.8	0, 0	0, 0	2.8
Total error	4.2, 1.8	5.3, 5.8	10.8, 4.8	2.6, 2.6	6.3, 2.4	5.6, 6.3	5.0, 1.3	7.1, 4.0	

[a] See Table 2, footnote *a*.
[b] Based on 216 tests.

TABLE 6. Comparison of disk elution test concentrations for each drug with the MIC_{50} and MIC_{90} of *B. fragilis* group isolates and the percent errors occurring with MICs near the disk test concentrations

Drug	Test concn (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Error (%)[a]
Cefoperazone	15	64	>128	63.2
Cefotaxime	18	16	64	95.7
Cefoxitin	18	8	32	71.4
Ceftazidime	18	32	>128	82.5
Ceftizoxime	18	16	64	76.8
Moxalactam	18	≤2	32	56.8
Piperacillin	60	8	64	16.7

[a] Within ±1 MIC increment of test concentration.

procedures. Table 6 depicts the relationship between the disk test concentrations, the MIC for 50% and 90% of the strains (MIC_{50} and MIC_{90}), and the errors with the *B. fragilis* group isolates. The MIC_{50}s and MIC_{90}s for cefotaxime, cefoxitin, ceftizoxime, and moxalactam were similar to the fixed test concentrations used in the disk elution methods. The errors among isolates having MICs within 1 concentration increment of the disk test concentrations of these drugs ranged from 56.8% (with moxalactam) to 95.7% (with cefotaxime). While the MIC_{50}s and MIC_{90}s were not proximate to the disk elution test concentrations of cefoperazone and ceftazidime, the majority of errors did occur with isolates having MICs near the fixed test concentration used for the disk tests (Table 6). Piperacillin MIC_{50}, MIC_{90}, and disk elution test concentrations also were similar, although far fewer overall classification errors occurred, including fewer errors in isolates with MICs near the disk test concentration. Approximately 45% of disk test errors in anaerobes other than the *B. fragilis* group occurred when MICs were close to the concentrations used for the disk tests.

Repeat testing of 10 *B. fragilis* group isolates and five of the drugs with the standard inoculum for each disk elution method and with carefully suspended and adjusted inoculum based on turbidity did not improve the accuracy of the four disk elution methods (Table 7). In addition, testing the same 10 *B. fragilis* isolates with twice the number of antibiotic disks also failed to reduce categorization errors by the disk elution methods (Table 7).

DISCUSSION

In the past several years, anaerobic bacteria have been shown to possess several mechanisms of antimicrobial resistance (5, 6, 8, 19, 24, 29, 35), including plasmid-mediated multiple drug resistance (16, 36). Several studies have demonstrated that anaerobes are no longer uniformly susceptible to drugs such as penicillin, chloramphenicol, clindamycin, and metronidazole (2, 3, 6, 7, 9, 34). Recently, a number of beta-lactam antibiotics have been marketed which have purportedly greater activity against anaerobes (22). However, susceptibility data have varied greatly between studies (14, 22, 25). Thus, susceptibility testing of individual anaerobe isolates may contribute significantly to the proper selection of antimicrobial agents for therapy. Until recently, routine susceptibility testing of anaerobes was discouraged, and clinician acceptance of susceptibility results seemed low (3, 9). Because of the changing susceptibility patterns and availability of newer drugs mentioned above, susceptibility results may now be in greater demand by physicians. A more recent recommendation suggests routine testing of anaerobe isolates recovered in pure culture or from documented bacteremia, central nervous system infections, and bone and joint infections (26).

Although the agar dilution method recommended by the NCCLS (21) offers very good reproducibility, few laboratories would choose this method for routine testing. Alternative methods which provide greater technical convenience include an abbreviated agar dilution method, broth microdilution, or broth disk elution techniques (P. R. Murray, Clin. Microbiol. Newsl. 7:113–116, 1985). A disk elution procedure may in fact be most practical for use in routine clinical laboratories for reasons of cost effectiveness and applicability to testing of even newer drugs.

The Schaedler, thioglycolate, and Wilkins-Chalgren broth disk methods were deemed the most convenient of the methods studied. The commercial availability of these media in either dehydrated form or prefilled tubes, as well as simple inoculation and incubation procedures, contributed to their overall facility. The recent deletion of the 2-h prediffusion period for the thioglycolate method (11) further enhances the convenience of that procedure. Both the PRAS BHI and Wilkins-West Vaspar overlay methods were more time-consuming and cumbersome because of their methods of obtaining anaerobiasis. In addition, determination of susceptibility by the allowance of turbidity aproaching 50% of that of the growth control with the PRAS BHI, thioglycolate, and Wilkins-West broths complicated the interpretation of a number of tests, especially those involving the *B. fragilis* group. However, the more rigorous requirement for inhibition of turbidity employed with the Schaedler and Wilkins-Chalgren broth tests did not provide greater accuracy than the more subjective endpoint interpretation described above. The disk elution methods heretofore have shown remarkable accuracy with older antimicrobial agents, including penicillin, chloramphenicol, clindamycin, and metronidazole (11, 13, 15, 37, 39). However, few data exist regarding

TABLE 7. Comparison of results obtained with 10 *B. fragilis* group isolates by reference and disk elution methods with varied inocula and numbers of disks

Method	% Full agreement[a] with:				% Essential agreement[a] with:			
	Standard no. of disks[b]		Twice standard no. of disks		Standard no. of disks		Twice standard no. of disks	
	Std.	Adj.	Std.	Adj.	Std.	Adj.	Std.	Adj.
PRAS BHI	76	70	62	66	90	86	90	92
Schaedler	67	60	73	64	87	78	91	93
Thioglycolate	76	54	80	62	96	74	94	94
Wilkins-Chalgren	80	72	68	64	94	90	88	92

[a] See the text for an explanation.
[b] See the text for the numbers of disks used with each drug. Std., Standard inoculum used; Adj., adjusted inoculum used.

the accuracy of these methods with more recently marketed beta-lactam antibiotics.

The present study indicates that several versions of the disk elution test provide reasonable accuracy for testing of many anaerobe species with cefoperazone, cefotaxime, ceftazidime, ceftizoxime, moxalactam, and piperacillin, as well as cefoxitin. Numerous errors were encountered with most of these drugs, however, when members of the *B. fragilis* group were tested. Only piperacillin and cefoxitin tests of these species yielded fewer than 10% errors. Our results agree with those of previous workers (1, 15), who noted a lack of reproducibility with isolates whose MICs were close to the single disk elution test concentration. Moxalactam also has been reported previously to yield inconsistent results in susceptibility tests with broth media (32). An additional factor may be variable diffusion of the different drugs from the disks. Perhaps our observation of trends of false susceptibility with cefoperazone and ceftazidime and false resistance with ceftizoxime relate to differing elution efficiencies of the drugs.

Errors encountered with the *B. fragilis* group in this study were not reduced either by greater attention to the inoculum density used for the tests, as had been suggested by others (4, 17, 18, 33), or by testing twofold-higher drug concentrations. This latter observation was made in response to the newest NCCLS proposal that several of these drugs be tested at higher fixed concentrations by the thioglycolate disk elution method (Murray, Clin. Microbiol. Newsl. 7:113–116, 1985; National Committee for Clinical Laboratory Standards, *Proposed Guidelines. Alternative Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. Proposed Standard M17-P*, in press). The NCCLS proposed guidelines recommend testing all drugs at a disk elution concentration which coincides with the highest MIC termed moderately susceptible according to criteria established in the NCCLS M7-T document (20). Thus, the recommended test concentrations in (micrograms per milliliter) are 60 for cefoperazone and piperacillin, 30 for cefotaxime, ceftizoxime, and moxalactam, and 18 for cefoxitin and ceftazidime. Testing at these concentrations may serve to detect only fully resistant isolates (since both moderately susceptible and susceptible isolates would be categorized the same). This represents a departure from the earlier descriptions of the disk elution methods which incorporated lower fixed test concentrations representing readily achievable drug levels to indicate organism susceptibility (13, 15, 39). Furthermore, the breakpoints described above coincide with the Food and Drug Administration breakpoints used by us only with cefoxitin, ceftazidime, and piperacillin. Published data supporting the efficacy of the disk elution test concentrations recommended by NCCLS have not appeared as yet. Our data suggest that testing of *B. fragilis* at higher concentrations does not serve to improve test accuracy. Moreover, errors of false susceptibility when testing at the resistance breakpoints of these drugs could create more serious errors in patient care.

The findings of this study support those of previous workers (11, 30) regarding the accuracy of cefoxitin and piperacillin results by disk elution testing, although Barry and Packer (1) expressed concern over a lack of reproducibility of the thioglycolate method with cefoxitin and cefotetan. Likewise, thioglycolate broth had been reported recently to be unsatisfactory for testing imipenem (30). Thus, further studies are needed to verify the accuracy of the thioglycolate method with newer beta-lactam antibiotics, especially in light of the higher test concentrations now recommended by the NCCLS (Murray, Clin. Microbiol. Newsl. 7:113–116, 1985).

We conclude that the disk elution methods described herein provided accurate results when applied to testing a variety of anaerobic bacteria with cefoxitin and piperacillin. However, errors encountered with certain anaerobes (particularly the *B. fragilis* group) and tests with cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and moxalactam cause us to recommend testing of these drugs by either the limited agar dilution (10; Murray, Clin. Microbiol. Newsl. 7:113–116, 1985) or perhaps a microdilution method (but not moxalactam by microdilution). Further improvements in the disk elution tests are needed to provide more accurate results with the *B. fragilis* group and the newer cephalosporins.

ACKNOWLEDGMENT

This study was supported in part by a grant from Lilly Research Laboratories.

LITERATURE CITED

1. **Barry, A. L., and R. R. Packer.** 1984. Determination of susceptibilty of anaerobic bacteria to cefotetan and cefoxitin by the thioglycolate disk elution method. J. Clin. Microbiol. **20**:912–916.
2. **Bawdon, R. E., E. Rozmiej, S. Palchaudhuri, and J. Krakowiak.** 1979. Variability in the susceptibility pattern of *Bacteroides fragilis* in four Detroit area hospitals. Antimicrob. Agents Chemother. **16**:664–666.
3. **Bourgault, A. M., J. L. Harkness, and J. E. Rosenblatt.** 1978. Clinical usefulness of susceptibility testing of anaerobes. Arch. Intern. Med. **138**:1825–1827.
4. **Bourgault, A.-M., and F. Lamothe.** 1984. Comparison of anaerobic susceptibility results obtained by two methods of inoculum preparation. J. Clin. Microbiol. **20**:1060–1064.
5. **Britz, M. L. and R. G. Wilkinson.** 1978. Chloramphenicol acetyltransferase of *Bacteroides fragilis*. Antimicrob. Agents Chemother. **14**:105–111.
6. **Britz, M. L., and R. G. Wilkinson.** 1979. Isolation and properties of metronidazole-resistant mutants of *Bacteroides fragilis*. Antimicrob. Agents Chemother. **16**:19–27.
7. **Cuchural, G. J., Jr., F. P. Tally, N. V. Jacobus, S. L. Gorbach, K. Aldridge, T. Cleary, S. M. Finegold, G. Hill, P. Iannini, J. P. O'Keefe, and C. Pierson.** 1984. Antimicrobial susceptibilities of 1,292 isolates of the *Bacteroides fragilis* group in the United States: comparison of 1981 with 1982. Antimicrob. Agents Chemother. **26**:145–148.
8. **Cuchural, G. J., Jr., F. P. Tally, N. V. Jacobus, P. K. Marsh, and J. W. Mayhew.** 1983. Cefoxitin inactivation by *Bacteroides fragilis*. Antimicrob. Agents Chemother. **24**:936–940.
9. **Finegold, S. M., and R. D. Rolfe.** 1983. Susceptibility testing of anaerobic bacteria. Diagn. Microbiol. Infect. Dis. **1**:33–40.
10. **Hauser, K. J., J. A. Johnston, and R. J. Zabransky.** 1975. Economical agar dilution technique for susceptibility testing of anaerobes. Antimicrob. Agents Chemother. **7**:712–714.
11. **Helstad, A. G., M. A. Hutchinson, W. P. Amos, and T. A. Kurzynski.** 1984. Evaluation of two broth disk methods for antibiotic susceptibility testing of anaerobes. Antimicrob. Agents Chemother. **26**:601–603.
12. **Jones, R. N., A. L. Barry, J. L. Cotton, V. L. Sutter, and J. M. Swenson.** 1982. Collaborative evaluation of the Micro-Media Systems anaerobe susceptibility panel: comparisons with reference methods and test reproducibility. J. Clin. Microbiol. **16**:245–249.
13. **Jorgensen, J. H., G. A. Alexander, and J. E. Johnson.** 1980. Practical anaerobic broth-disk elution susceptibility test. Antimicrob. Agents Chemother. **17**:740–742.
14. **Jorgensen, J. H., S. A. Crawford, and G. A. Alexander.** 1980. Comparison of moxalactam (LY127935) and cefotaxime against anaerobic bacteria. Antimicrob. Agents Chemother. **17**:

901–904.
15. **Kurzynski, T. A., J. W. Yrios, A. G. Helstad, and C. R. Field.** 1976. Aerobically incubated thioglycolate broth disk method for antibiotic susceptibility testing of anaerobes. Antimicrob. Agents Chemother. **10**:727–732.
16. **Malamy, M. H., and F. P. Tally.** 1981. Mechanisms of drug resistance transfer in *Bacteroides fragilis*. J. Antimicrob. Chemother. **8**(Suppl. D):59–75.
17. **Mangels, J. I., and L. H. Lindberg.** 1985. Evaluation of broth microdilution susceptibility results for anaerobic organisms by use of a rapid direct colony inoculum. J. Clin. Microbiol. **21**:269–272.
18. **Murray, P. R., and A. C. Niles.** 1983. Inoculum preparation for anaerobic susceptibility tests. J. Clin Microbiol. **18**:733–734.
19. **Murray, P. R., and J. E. Rosenblatt.** 1977. Penicillin resistance and penicillinase production in clinical isolates of *Bacteroides melaninogenicus*. Antimicrob. Agents Chemother. **11**:605–608.
20. **National Committee for Clinical Laboratory Standards.** 1983. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Tentative standard, M7-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
21. **National Committee for Clinical Laboratory Standards.** 1985. Reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard. M11-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
22. **Neu, H. C.** 1983. Structure-activity relations of new β-lactam compounds and in vitro activity against common bacteria. Rev. Infect. Dis. **5**(Suppl.):S319–S337.
23. **Polomski, J. C., S. H. Bauer, and K. D. McClatchey.** 1983. Micro-Media Systems anaerobe panel versus broth disk method in anaerobe antimicrobial testing. J. Clin. Microbiol. **17**: 949–952.
24. **Privitera, G., F. Fayolle, and M. Sebald.** 1981. Resistance to tetracycline, erythromycin and clindamycin in the *Bacteroides fragilis* group. Inducible versus constitutive tetracycline resistance. Nature (London) **20**:314–320.
25. **Rolfe, R. D. and S. M. Finegold.** 1981. Comparative in vitro activity of new beta-lactam antibiotics against anaerobic bacteria. Antimicrob. Agents Chemother. **20**:600–609.
26. **Rosenblatt, J. E.** 1984. Antimicrobial susceptibility testing of anaerobic bacteria. Rev. Infect. Dis. **6**:S242–S248.
27. **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.
28. **Salaki, J. S., R. Black, F. P. Tally, and J. W. Kislak.** 1976. *Bacteroides fragilis* resistant to the administration of clindamycin. Am. J. Med. **60**:83–88.
29. **Sato, K., Y. Matsuura, M. Inoue, and S. Mitushashi.** 1982. Properties of a new penicillinase type produced by *Bacteroides fragilis*. Antimicrob. Agents Chemother. **22**:579–584.
30. **Shungu, D. L., E. Weinberg, and A. T. Cerami.** 1985. Evaluation of three broth disk methods for testing the susceptibility of anaerobic bacteria to imipenem. J. Clin. Microbiol. **21**:875–879.
31. **Sosa, A., F. P. Tally, N. V. Jacobus, and S. L. Gorbach.** 1982. *Bacteroides fragilis* resistance to clindamycin in vitro. Antimicrob. Agents Chemother. **22**:771–774.
32. **Sutter, V. L., J. Emmerman, E. Randall, R. J. Zabransky, and R. J. Birk.** 1985. Establishment of MICs of moxalactam for control and reference anaerobic organisms in agar dilution and microdilution techniques. Antimicrob. Agents Chemother. **27**:424–426.
33. **Swenson, J. M., and C. Thornsberry.** 1984. Preparing inoculum for susceptibility testing of anaerobes. J. Clin. Microbiol. **19**:321–325.
34. **Tally, F. P., G. J. Cuchural, N. V. Jacobus, S. L. Gorbach, K. E. Aldridge, T. J. Cleary, S. M. Finegold, G. B. Hill, P. B. Iannini, R. V. McCloskey, J. P. O'Keefe, and C. L. Pierson.** 1983. Susceptibility of the *Bacteroides fragilis* group in the United States in 1981. Antimicrob. Agents Chemother. **23**:536–540.
35. **Tally, F. P., J. P. O'Keefe, N. M. Sullivan, and S. L. Gorbach.** 1979. Inactivation of cephalosporins by *Bacteroides*. Antimicrob. Agents Chemother. **16**:565–571.
36. **Tally, F. P., D. R. Snydman, S. L. Gorbach, and M. H. Malamy.** 1979. Plasmid-mediated transferable resistance to clindamycin and erythromycin in *Bacteroides fragilis*. J. Infect. Dis. **139**:83–88.
37. **West, S. E. H., and T. D. Wilkins.** 1980. Vaspar broth-disk procedure for antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. **17**:288–291.
38. **Wilkins, T. D., and S. Chalgren.** 1976. Medium for use in antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. **10**:926–928.
39. **Wilkins, T.D., and T. Thiel.** 1973. Modified broth-disk method for testing the antibiotic susceptibility of anaerobic bacteria. Antimicrob. Agents Chemother. **3**:350–356.