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Evaluation of Two Broth Disk Methods for Antibiotic Susceptibility Testing of Anaerobes

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We evaluated the aerobic thioglycolate broth disk and the vaspar overlay broth disk methods for antibiotic susceptibility testing of 144 strains of anaerobes. For penicillin, carbenicillin, chloramphenicol, and metronidazale, both broth disk methods yielded at least 95% agreement with results obtained by the National Committee for Clinical Laboratory Standards reference agar dilution procedure. For cefoxitin and clindamycin, the agreement was ca. 90%. Overall, the aerobic thioglycolate broth disk and vaspar overlay broth disk methods yielded agreements of 93.3 and 93%, respectively, with the National Committee for Clinical Laboratory Standards method.

Recent reports of increased incidence of antibiotic resistance in anaerobes raise the question of a possible need for routine antibiotic susceptibility testing of these pathogens (1, 3, 6). Bourgault et al. (2) have also suggested that antibiotic susceptibility testing of anaerobes is particularly important in bacteremias, orthopedic infections, and brain abscesses.

We are reporting the results of our evaluation of two practical antibiotic susceptibility methods for anaerobes: the aerobically incubated thioglycolate broth disk method (AeTBD) of Kurzynski et al. (5) and the vaspar overlay broth disk method (VBD) of West and Wilkins (12). The AeTBD and VBD methods were compared with the reference agar dilution procedure proposed by the National Committee for Clinical Laboratory Standards (NCCLS) (8, 11). A total of 144 clinical isolates which were submitted to our laboratory between July 1980 and December 1980 were used. Most of the isolates were submitted by the following laboratories: the Center for Health Sciences, University of Wisconsin, Madison, Wis.; Bellin Memorial Hospital, Green Bay, Wis.; and Marshfield Medical Center, Marshfield, Wis. The genera, species, and numbers of anaerobes submitted were: *Bacteroides distasonis*, 5; *Bacteroides fragilis*, 24; *Bacteroides melaninogenicus*, 4; *Bacteroides thetaiotaomicron*, 9; *Bacteroides vulgatus*, 5; other *Bacteroides* species, 7; *Clostridium perfringens*, 23; other *Clostridium* species, 23; *Eubacterium lentum*, 5; *Fusobacterium nucleatum*, 9; other *Fusobacterium* species, 7; *Peptococcus asaccharolyticus*, 2; *Peptococcus magnus*, 8; *Peptococcus prevotii*, 1; *Peptostreptococcus anaerobius*, 2; *Peptostreptococcus micros*, 6; and *Veillonella parvula*, 4. All isolates were identified at the State Laboratory of Hygiene by methods described in the Anaerobe Laboratory Manual (4). Cultures were stored in chopped meat carbohydrate broth at −70°C and subcultured once to plates of brain heart infusion-supplemented agar (5). The NCCLS procedure recommends two to three sequential transfers to ensure reproducible results (8).

The NCCLS reference agar dilution procedure was performed as originally described (8) with a Steers replicator for inoculation (10). In tests with isolates of *B. melaninogenicus*, 5% defibrinated sheep blood was added to Wilkins-Chalgren agar (13).

The AeTBD method was performed as originally described by Kurzynski et al. (5), with thioglycolate both in 5-ml quantities. Rabbit serum containing hemin and menadione was used routinely as a supplement in tests with *B. melaninogenicus* (5). The serum was found to be essential for good growth. Some isolates of *Bacteroides ruminicola*, *Bacteroides bivius*, and *E. lentum* also needed serum supplementation in the AeTBD and VBD methods.

For the VBD method, Wilkins-West broth (13) was used in 10-ml volumes dispensed into screw-capped tubes (16 by 125 mm). For isolates of *B. melaninogenicus*, 10% rabbit serum was required. Overnight chopped meat carbohydrate cultures were used as inoculum sources in each broth disk method. Table 1 lists the number of antibiotic disks and resultant antibiotic concentrations for both broth disk methods.

All antibiotic disks except metronidazole (G. D. Searle and Co., Chicago, Ill.) were supplied by Difco Laboratories, Detroit, Mich. Disks were stored in the manner recommended by the manufacturers (usually at 2 to 8°C). Quality control of the disks was done by the disk diffusion method with *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aruginosa* ATCC 27853 (7).

Seven reference standard antibiotic powders were used: penicillin G (Bristol Laboratories, Syracuse, N.Y.); carbenicillin (Pfizer Inc., New York, N.Y.); cefoxitin (Merck Sharp & Dohme, West Point, Pa.); tetracycline (Bristol Laboratories, Syracuse, N.Y.); chloramphenicol (Parke, Davis & Co., Detroit, Mich.); metronidazole (G. D. Searle and Co., Chicago, Ill.); and clindamycin (Upjohn Co., Kalamazoo, Mich.). Stock concentrations of antibiotics were prepared at 1,280 μg/ml (except carbenicillin, which was 5,120 μg/ml) and kept frozen at −70°C for up to 4 months.

When there was disagreement between methods, Gram-stained smears were examined to determine purity of cultures, and the tests were repeated.

Table 2 shows the agreement of antibiotic susceptibility test results obtained by testing 144 anaerobes with two broth disk methods and the NCCLS reference agar dilution procedure. Results were interpreted as being in agreement when an isolate yielded the same susceptible or resistant interpretation by the broth disk and agar dilution procedures. Both broth disk methods gave at least 95% agreement of results with the agar dilution procedure when testing the anaerobes against penicillin, carbenicillin, metronidazole, and chloramphenicol. Cefoxitin and clindamycin broth disk method results agree with those of the agar dilution procedure ca.

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TABLE 1. Antibiotic disks used and antibiotic concentrations obtained in the AeTBD and VBD methods

Antibiotic	Labeled disk content	No. of disks per tube		Calculated test concn per ml
		AeTBD	VBD	
Penicillin G	10 U	1	2	2 U
Carbenicillin	100 μg	5	10	100 μg
Cefoxitin	30 μg	3	6	18 μg
Chloramphenicol	30 μg	2	4	12 μg
Clindamycin	2 μg	4	8	1.6 μg
Tetracycline	30 μg	1	2	6 μg
Metronidazole	50 μg	1	2	10 μg

90% of the time. With tetracycline, the agreement was 86% for the VBD method and 90% for the AeTBD method.

There were 27 instances in which cefoxitin broth disk tests disagreed with the agar dilution procedure. Of the 21 (78%) of these which were false susceptible readings, 9 were by the AeTBD and 12 were by the VBD method. All of the 21 false susceptible readings involved 12 isolates of the *B. fragilis* group, and in each case the MIC was 32 μg/ml, or one twofold dilution above the susceptibility breakpoint. Similar results were obtained with clindamycin, for which there were 29 instances of disagreement, 15 with the AeTBD and 14 with the VBD method. Of these 29 disagreements, 22 (76%) were false susceptible readings, and for 21 of these (12 isolates), the MIC was 4 μg/ml, which is one twofold dilution above the susceptible breakpoint. Our laboratory has previously noted that false susceptible AeTBD results most often occur when MICs are close to the breakpoint value (5).

With tetracycline, there were 35 disagreements, 15 with the AeTBD and 20 with the VBD method. Of these disagreements, 29 (83%) were false susceptible readings. Unlike cefoxitin and clindamycin, only nine (26%) of the readings were within one twofold dilution of the susceptible breakpoint. The problem in obtaining agreement between the broth disk methods and the agar dilution procedure in tests with tetracycline has been discussed in other studies (5, 9, 14).

For the 1,008 tests performed by each broth disk method, there was 93.3% agreement between the results obtained by the AeTBD and agar dilution procedures and 93.0% agreement between the VBD and agar dilution procedures. False susceptible readings were far more common than false resistant ones: 95 and 44, respectively. Overall, there were 39 (3.8%) false susceptible readings and 29 (2.9%) false resistant readings with the AeTBD method. The VBD method produced 56 (5.6%) false susceptible readings and 15 (1.5%) false resistant readings.

Both broth disk methods are much easier to perform and less time consuming than the reference agar dilution method, and they yield equally reliable results. The VBD method has an advantage over the AeTBD in that a prediffusion time is not needed before inoculation. However, as a result of testing 37 common anaerobes versus all seven antibiotics used in this study (259 tests), we have determined that the preincubation time is not necessary for the AeTBD method (unpublished data), and we have eliminated it. The AeTBD advantages are that thioglycolate broth is commercially available and easy to prepare (Wilkins-West broth is not), the amounts of medium and disks are one-half of those used in the VBD method, and no vaspar plug is necessary. We have also confirmed that some isolates of *B. melaninogenicus* will not grow in Wilkins-West broth (13) unless 10% serum is added. Since growth of the anaerobes that we tested was very comparable in both the AeTBD and VBD methods, and in view of the advantages noted above, we prefer the AeTBD method.

TABLE 2. Agreement between antibiotic susceptibility results obtained with the AeTBD and VBD methods and those obtained with the NCCLS reference agar dilution method

Antibiotic	No. (%) of results agreeing with the agar dilution result[a]	
	AeTBD	VBD
Pencillin G	138 (95.8)	139 (96.5)
Carbenicillin	139 (96.5)	140 (97.2)
Metronidazole	137 (95.1)	137 (95.1)
Chloramphenicol	137 (95.1)	137 (95.1)
Cefoxitin	131 (91.0)	130 (90.3)
Tetracycline	129 (89.6)	124 (86.1)
Clindamycin	129 (89.6)	130 (90.3)
Overall % agreement	93.3	93.0

[a] Results from 144 anaerobes tested.

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