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Evaluation of the E Test for Susceptibility Testing of Anaerobic Bacteria

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The susceptibilities of 105 clinical isolates of anaerobic bacteria were determined by a new method, the E test (AB Biodisk, Solna, Sweden), and were compared with the MICs for these organisms obtained by the reference agar dilution method by using supplemented brucella and Wilkins-Chalgren agars. The E test is a plastic strip with a predefined antibiotic gradient immobilized on one side and a MIC interpretive scale printed on the other side. Strips with cefoxitin, cefotaxime, imipenem, penicillin, metronidazole, and clindamycin were used in this study. A suspension of the test strain equal to the visual turbidity of a no. 0.5 McFarland standard was prepared and swabbed onto a 150-mm-diameter plate. The strips were applied in a radial fashion, and the plates were incubated under anaerobic conditions. After growth had occurred, an ellipse of inhibition was seen around each strip. At the point of intersection of the ellipse with the strip, the MIC was read from the interpretive scale. For most antibiotic-organism combinations, the ellipse was clear and the endpoint was sharp. The E-test MICs were interpreted after overnight and 48-h incubation for 58 of the strains. After overnight incubation, 87% of the E-test MICs were within 1 dilution of the agar dilution MICs, and 98% were within 2 dilutions. After 48 h of incubation, agreement was 86 and 97% respectively. E-test MICs obtained for the *Bacteroides fragilis* group after overnight incubation were more comparable than those obtained after 48 h of incubation to agar dilution MICs determined at 48 h for all drugs except clindamycin. On brucella agar, there was a 2% categorical discrepancy rate between the E-test MICs and agar dilution MICs, which occurred mostly with cefoxitin. The E test is easy to perform and read, is suitable for all anaerobes, can be used to test single patient isolates as needed, and offers the laboratory a reliable method for susceptibility testing of anaerobic bacteria.

The National Committee for Clinical Laboratory Standards (NCCLS) (16) guidelines for susceptibility testing of anaerobic bacteria recommend susceptibility testing of individual patient isolates under certain circumstances. The reference agar dilution method is not readily adaptable for testing individual isolates, and the broth microdilution method has drawbacks because of discrepancies with the reference method (1) and because many anaerobes do not grow well in broth media (16). The modified broth disk elution method, a 24-h test that is convenient for testing individual isolates (14), has been demonstrated to be inaccurate for testing the activity of beta-lactam agents against members of the *Bacteroides fragilis* group (13, 21) and is no longer recommended by NCCLS as an acceptable procedure (16). Although patients with anaerobic infections are usually treated empirically (7, 8), in certain situations, such as therapy for chronic infections, undrainable abscesses, bacteremia, or failure of initial therapy, susceptibility testing is recommended. A simple, rapid, and reliable method that can be used to test individual isolates in these circumstances is needed.

The E test (AB Biodisk, Solna Sweden) is a plastic strip (5 by 50 mm; antibiotic carrier) with a continuous gradient of antibiotic immobilized on one side and an MIC interpretive scale corresponding to 15 twofold MIC dilutions on the other side. The susceptibility test is performed by placing the strip on the surface of an inoculated agar plate. After incubation, an ellipse of inhibition is formed around the strip, and at the point where the ellipse intersects the strip, the MIC of the antibiotic is read from the interpretive scale. It has been found to be a reliable method for determining the susceptibilities of fastidious and other aerobic and facultatively anaerobic bacteria (2, 4, 7, 10, 12). In this study we evaluated this method for susceptibility testing of clinical isolates of anaerobic bacteria.

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MATERIALS AND METHODS

Strains. One hundred five clinical isolates of anaerobic bacteria were tested. These organisms were selected because of growth characteristics and previous susceptibility results to provide a comprehensive challenge to the method. They were *Bacteroides fragilis* (8 strains), *Bacteroides thetaiotaomicron* (7 strains), *Bacteroides distasonis* (8 strains), *Bacteroides vulgatus* (8 strains), *Bacteroides ovatus* (8 strains), *Bacteroides uniformis* (8 strains), other *Bacteroides* spp. (4 strains), *Wolinella* sp. (1 strain), *Prevotella* spp. (9 strains), *Porphyromonas* spp. (3 strains), *Fusobacterium mortiferum* and *F. varium* (7 strains), *Fusobacterium nucleatum* and *F. necrophorum* (4 strains), *Peptostreptococcus* spp. (10 strains), *Clostridium perfringens* (4 strains), other *Clostridium* spp. (8 strains), and non-spore-forming gram-positive rods (8 strains). The organisms were identified by standard methods (17). Five reference strains were included: *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *B. vulgatus* ATCC 29327, *C. perfringens* ATCC 13124, and

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Peptostreptococcus magnus ATCC 29328. All organisms were maintained at −70°C in 10% skim milk. They were subcultured at least twice on PRAS brucella blood agar (Anaerobe Systems, San Jose, Calif.) prior to testing.

Antimicrobial agents. The following antibiotics, which were available on the prototype E-test strips, were tested: cefoxitin, cefotaxime, imipenem, penicillin, metronidazole, and clindamycin. The E-test strips and laboratory standard powders were supplied by AB Biodisk. The antibiotic powders were reconstituted according to the instructions of the manufacturer and were used on the day of the test.

Agar dilution MIC determinations. MICs were determined by the NCCLS reference agar dilution method (16) by using brucella agar (Difco Laboratories, Detroit, Mich.) supplemented with vitamin K, hemin, and 5% laked sheep blood (BBA) and Wilkins-Chalgren agar (Difco) without added blood (WCA), as recommended by NCCLS. BBA and WCA plates containing serial dilutions of the antimicrobial agents ranging from 256 to 0.015 μg/ml were prepared and used on the same day. The inoculum was prepared in an anaerobic chamber by suspending several colonies from a 72-h culture plate into brucella broth to achieve the visual turbidity of a no. 0.5 McFarland standard. A Steers replicator (Craft Machine Co. Inc., Chester, Pa.) was used to apply the organisms to the plates for a final inoculum of 10^5 cfu per spot. Plates were incubated in anaerobic jars with GasPak (BBL, Cockeysville, MD) for 48 h. The MIC was defined as the lowest concentration of antibiotic that resulted in no growth, a barely visible haze, or one colony.

E test. The E test was evaluated by using BBA and WCA in 150-mm-diameter plates containing 60 to 65 ml of agar. The plates were prepared in-house. The inoculum for the E test was the same as that used for the agar dilution tests. This was approximately 10^8 CFU/ml, as described previously for disk diffusion testing of anaerobes (3, 9, 18). The inoculum was applied with a sterile cotton swab, using a fresh swab for each plate. The antibiotic carriers were then applied in a radial pattern, with six strips applied to each plate. Inoculation of the plates was performed outside the anaerobic chamber. Plates were incubated in an incubator within an anaerobic chamber (Anaerobe Systems) or in anaerobic jars at 36°C. The E-test plates incubated in the anaerobic chamber were evaluated after incubation overnight (approximately 20 h) and for 48 h, and those in jars were examined only after 48 h of incubation. After incubation, an elliptical zone of growth inhibition was seen around the strip. The MIC was read from the scale at the intersection of the zone with the strip. The E-test MICs that were between the standard twofold dilution steps were rounded to the next higher step for comparison with agar dilution MICs. The principle of the E test is illustrated in Fig. 1.

A categorical discrepancy was defined as a ≥2-dilution difference that resulted in a change of interpretation from susceptible to resistant or vice versa. The breakpoints (in micrograms per milliliter) used for interpretation as susceptible were as follows: cefoxitin, ≤32; cefotaxime, ≤32; imipenem, ≤8; penicillin, ≤2; metronidazole, ≤16; and clindamycin, ≤4 (16). (The activity of penicillin on the E-test strips is expressed in micrograms per milliliter, and the breakpoint has been adjusted accordingly).

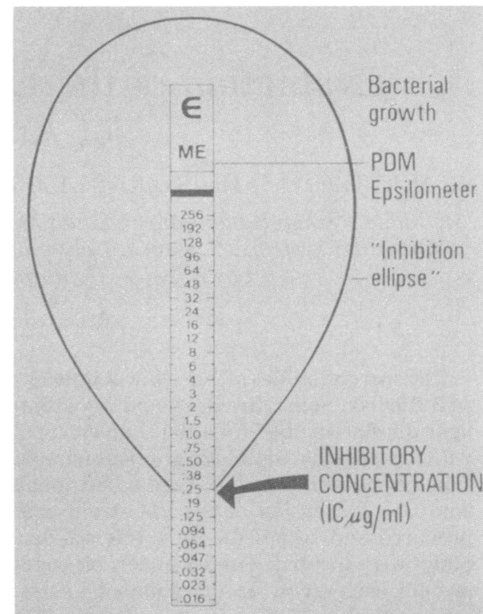

FIG. 1. The E-test principle. The E test uses a strip coated with a logarithmic gradient of an antimicrobial agent applied to an inoculated plate. After incubation, an ellipse of inhibition is formed. At the intersection of the ellipse with the strip, the MIC is read from the interpretive scale.

RESULTS

E-test MICs were read after overnight incubation for 68 of the strains, including 51 strains of the *B. fragilis* group and clostridia plus 17 other slower-growing strains. The reading at 48 h included all strains that grew and that had on-scale endpoints. The E-test MIC was compared with the MIC obtained by agar dilution with the same agar. A comparison of the overnight and 48-h E-test MICs versus the 48-h agar dilution MICs for all organisms tested is presented in Tables 1 and 2. The E-test MICs were easy to read and had clear, sharp endpoints for most of the organism-antibiotic combinations. For β-lactamase-producing strains, in some instances, there was haziness and pinpoint growth that obliterated the ellipse. This occurred mostly with some members of the *B. fragilis* group with cefoxitin on WCA. Figure 2 depicts E-test MIC determinations for *B. thetaiotaomicron*.

Most of the strains of clostridia grew and displayed clear endpoints after overnight incubation, with little change in the MIC after additional incubation. After 48 h of incubation, 59 of 68 (87%) of E-test MICs determined on BBA and WCA were within 1 dilution of the agar dilution MICs. There was a tendency for imipenem, metronidazole, and penicillin MICs determined by agar dilution to be about 1 dilution higher. Since the MICs were very low, however, this did not change the interpretive result. Clindamycin E-test MICs increased by 1 dilution after day 2 of incubation for 4 of the 12 strains. For six strains, cefoxitin agar dilution MICs ranged from 32 to 128 μg/ml. For five of these strains, E-test MICs were consistently 1 to 2 dilutions higher than the agar dilution MICs, although this did not result in any interpretive discrepancies. This occurred on both agars.

Of the non-*B. fragilis* group *Bacteroides* spp., *Prevotella* spp., and *Porphyromonas* spp., some strains were very susceptible to the agents tested, and the MICs determined by both methods were off-scale. Some of the strains grew poorly or not at all on WCA, and MICs determined on WCA were generally 1 to 2 dilutions lower than MICs determined on BBA. Of the strains for which the MICs were on-scale, the agreement was generally good with no categorical dis-

TABLE 1. Comparison of E-test MICs determined after overnight incubation with agar dilution MICs for all anaerobic strains

Antibiotic	Medium	No. of strains[a]	% of E-test MICs within the following concentration (log_2) agar dilution MIC:							% within ±1 dilution	No. of categorical discrepancies
			>−2	−2	−1	Same	+1	+2	>+2		
Cefoxitin	BBA	54	0	2	26	48	19	4	2	93	2
	WCA	56	2	4	9	36	27	18	5	72	10
Ceftizoxime	BBA	49	8	6	18	51	14	2	0	83	1
	WCA	53	9	8	25	36	21	2	0	82	1
Imipenem	BBA	49	2	10	45	41	2	0	0	88	0
	WCA	52	0	8	21	63	8	0	0	92	0
Penicillin G	BBA	56	0	7	27	59	7	0	0	93	0
	WCA	52	0	8	13	54	17	6	2	84	0
Metronidazole	BBA	54	0	7	26	50	13	4	0	89	0
	WCA	56	0	5	34	45	16	0	0	95	0
Clindamycin	BBA	45	4	20	27	47	2	0	0	74	2
	WCA	49	10	16	37	33	4	0	0	74	2
All	BBA	307	2	9	28	49	10	10	0	87	5
	WCA	318	3	8	23	45	16	4	1	83	13

[a] Number of strains for which MICs were finite.

crepancies. After 48 h of incubation, MICs for 58 of 66 (85%) of the tests determined on BBA and MICs for 40 of 49 (82%) of the tests determined on WCA were within 1 dilution of the MICs determined by agar dilution.

The fusobacteria exhibited a tailing phenomenon for the agar dilution MICs with the beta-lactam antibiotics that made reading of endpoints of growth somewhat ambiguous. In most cases in the E-test MIC determinations, the strains exhibited an area of heavy growth with good demarcation of the ellipse and then a haze extending into the ellipse. Examination of a Gram stain of this haze revealed gram-negative bizarre forms and debris suggestive of L forms. The change from haze to real growth was easily seen by the E test, while agar dilution MIC endpoints were more difficult to interpret. After 48 h of incubation, 36 of 46 (78%) of the E tests on BBA and 36 of 48 (75%) of the E tests on WCA were within 1 dilution of the MICs determined by agar dilution. One major discrepancy occurred with a β-lactamase-producing strain of *F. nucleatum* for which the penicillin agar dilution MIC was 8 to 16 μg/ml and the E-test MIC was 1 μg/ml. The test was repeated several times, with the same result.

For the non-spore-forming gram-positive bacilli, agar dilution and E-test MICs showed good agreement. Two strains

TABLE 2. Comparison of E-test MICs determined after 48 h of incubation with agar dilution MICs for all anaerobic strains

Antibiotic	Medium	No. of strains[a]	% of E-test MICs within the following concentration (log_2) of agar dilution MIC:							% within ±1 dilution	No. of categorical discrepancies
			>−2	−2	−1	Same	+1	+2	>+2		
Cefoxitin	BBA	91	1	3	25	40	20	9	2	85	7
	WCA	90	2	7	17	29	19	10	17	65	17
Cefotaxime	BBA	91	4	11	16	45	20	2	1	81	1
	WCA	89	3	5	18	39	20	14	1	77	4
Imipenem	BBA	81	2	19	46	31	2	0	0	79	0
	WCA	78	1	10	32	49	9	0	0	90	0
Penicillin G	BBA	89	3	6	35	44	12	0	0	91	2
	WCA	85	2	7	18	51	18	4	1	87	1
Metronidazole	BBA	92	1	16	30	43	5	2	1	78	0
	WCA	85	0	16	40	34	6	4	0	80	0
Clindamycin	BBA	79	0	1	10	58	28	1	1	96	2
	WCA	77	0	5	26	52	12	5	0	90	2
All	BBA	523	2	9	27	44	15	1	1	86	12
	WCA	504	1	8	25	42	14	6	3	81	24

[a] Number of strains for which MICs were finite.

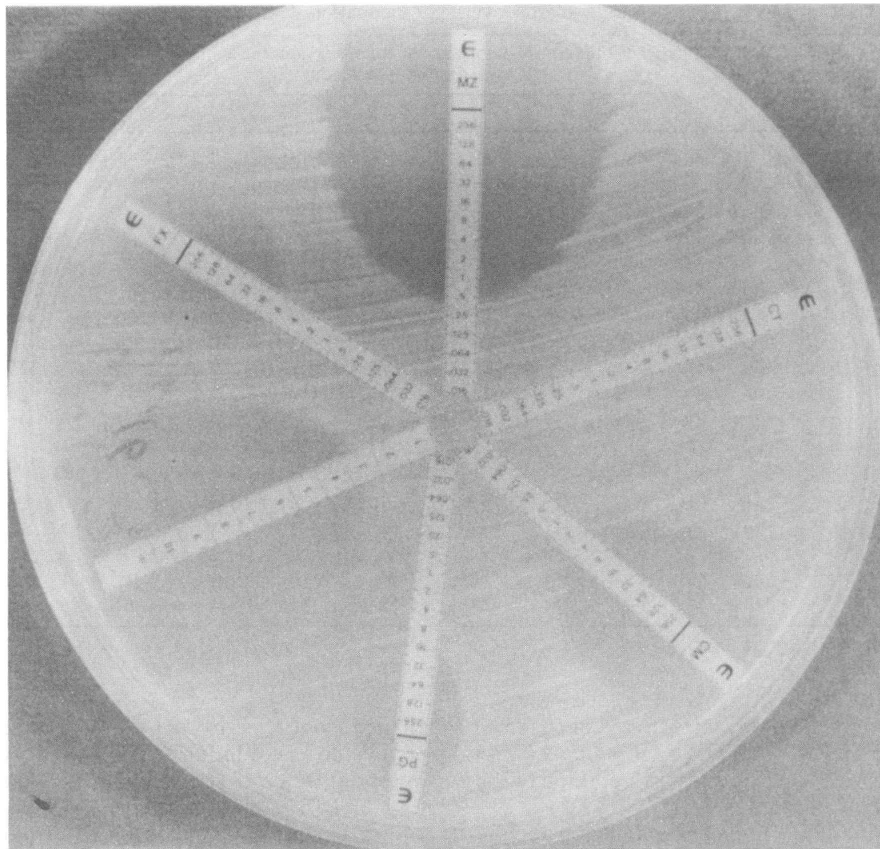

FIG. 2. Susceptibility of *B. thetaiotaomicron* to metronidazole (MZ), cefotaxime (CT), clindamycin (CM), penicillin G (PG), imipenem (IP), and cefoxitin (CX) determined by the E test.

of *Propionibacterium acnes* were too susceptible to most of the antimicrobial agents for comparison. For the strains that grew after overnight incubation, there was little increase in the MIC after additional incubation. Five of these strains were at least moderately resistant to metronidazole, and there was a good correlation in the MICs obtained by the two methods, with 13 of 15 (87%) of MIC determinations being within 1 dilution of each other. Overall, at 48 h of incubation, 36 of 40 (90%) of E-test MICs determined on BBA and 35 of 40 (88%) E-test MICs determined on WCA were within 1 dilution of the agar dilution MICs. One categorical discrepancy occurred with one strain of *Eubacterium limosum* for which the cefoxitin agar dilution MIC was 32 μg/ml and for which the E-test MIC was 128 μg/ml after overnight incubation but that increased to 256 μg/ml after 48 h of incubation.

There was good agreement between the methods for peptostreptococci. After 48 h of incubation, 28 of 35 (80%) of E-test MICs on BBA and 26 of 31 (84%) E-test MICs on WCA were within 1 dilution of the MICs determined by agar dilution. Metronidazole MICs determined by the E test were 2 dilutions lower than the agar dilution MICs for three strains and were 3 dilutions lower for a fourth strain. This did not result in a categorical discrepancy.

Most of the *B. fragilis* group grew after overnight incubation and yielded readable endpoints. The comparison of E-test MICs obtained after overnight incubation with agar dilution MICs is presented in Table 3. The ±1-dilution agreement between the methods for imipenem, penicillin, and metronidazole was very good after both overnight and 48-h incubation, with a tendency for E-test MICs to be lower. When the E-test clindamycin MICs obtained after overnight incubation were compared with the agar dilution MICs, only 61 to 69% were within 1 dilution; however, after 48 h of incubation, agreement was 98% on BBA and 90% on WCA. The E-test MICs were several dilutions lower than the agar dilution MICs. To clarify this observation, 15 strains that exhibited this discrepancy were selected and MIC determinations were repeated by both methods, except that the agar dilution MICs were also interpreted after overnight incubation and again after 48 h of incubation to allow a comparison between the methods and to determine whether the agar dilution MICs also increased with continued incubation. The agar dilution MICs determined after overnight incubation were 1 to 4 dilutions lower than the MICs measured after 48 h. When the overnight E-test MICs were compared with the overnight agar dilution MICs, agreement was improved between the methods, with 13 of 15 (87%) of the previously discrepant MICs being within 1 dilution. There was little difference noted for cefoxitin and penicillin agar dilution MICs measured after overnight and 48-h incubation. For 2 of 30 strains, there was a fourfold increase in the cefotaxime MIC between overnight and 48-h incubations. For cefoxitin, agreement between the methods was very good when BBA was used. After overnight incubation, 29 of 31 (94%) of E-test MICs were within 1 dilution of the agar dilution MICs. After 48 h, 37 of 45 (82%) E-test MICs were within 1 dilution, with the E-test MICs showing a tendency to increase with prolonged incubation. Two strains

TABLE 3. Comparison of E-test MICs determined after overnight incubation with agar dilution MICs for the *B. fragilis* group

Antibiotic	Medium	No. of strains[a]	% of E-test MICs within the following concentration (log_2) of agar dilution MIC:							% within ±1 dilution	No. of categorical discrepancies
			>−2	−2	−1	Same	+1	+2	>+2		
Cefoxitin	BBA	33	0	0	18	61	15	3	3	94	2
	WCA	38	3	3	11	26	32	18	8	69	9
Cefotaxime	BBA	33	9	3	18	45	15	3	0	78	1
	WCA	38	5	8	26	39	18	3	0	83	1
Imipenem	BBA	33	3	9	45	42	0	0	0	87	0
	WCA	39	0	5	23	64	8	0	0	95	0
Penicillin G	BBA	33	0	6	12	72	9	0	0	93	0
	WCA	37	0	8	11	51	22	8	3	84	0
Metronidazole	BBA	35	0	3	33	54	11	0	0	98	0
	WCA	38	0	5	34	47	13	0	0	94	0
Clindamycin	BBA	28	11	29	32	29	0	0	0	61	2
	WCA	30	17	13	33	33	3	0	0	69	2
All	BBA	185	4	8	26	51	8	1	1	85	5
	WCA	210	4	7	23	43	16	5	2	82	12

[a] Number of strains for which MICs were finite.

yielded discrepant results. For both strains, agar dilution MICs were 32 μg/ml, and E-test MICs were 128 and 256 μg/ml after overnight incubation. After 48 h of incubation, four additional strains for which the agar dilution MICs were 16 to 32 μg/ml, the E-test MICs were 64 to 256 μg/ml. Cefoxitin MICs determined on WCA had a lower correlation between the methods. After overnight incubation, 27 of 38 (69%) of E-test MICs were within 1 dilution of the agar dilution MICs. After 48 h of incubation, 26 of 45 (58%) were within 1 dilution. Nine strains yielded higher E-test MICs after overnight incubation, and after 48 h of incubation, 16 strains yielded higher E-test MICs than agar dilution MICs, resulting in categorical discrepancies. Cefotaxime yielded acceptable agreement between the methods. After overnight incubation, 78% of E-test MICs on BBA and 83% of E-test MICs on WCA were within 1 dilution of the agar dilution MICs, with one categorical discrepancy on each medium. At 48 h, 34 of 45 (76%) E-test MICs on BBA and 30 of 45 (67%) E-test MICs on WCA were within 1 dilution. At 48 h, two categorical discrepancies occurred on BBA and three occurred on WCA.

The American Type Culture Collection control strains were tested at least five times with each antibiotic and on each agar. The reproducibility of the E test was 528 of 539 tests (98%) within a 2-dilution interval and 100% within a 3-dilution interval. In comparison with agar dilution tests with penicillin, metronidazole, imipenem, and clindamycin, overnight E-test MICs exhibited 94% agreement within 1 dilution, with no interpretive discrepancies. *B. thetaiotaomicron* yielded discrepant results when it was tested with cefoxitin and cefotaxime on WCA. Cefoxitin E-test MICs were 1 to 3 dilutions higher than the agar dilution MICs. The mode E-test MIC of cefoxitin was 64 μg/ml after overnight incubation and 256 μg/ml after 48 h of incubation. The acceptable range is 8 to 32 μg/ml. E-test MICs of cefotaxime were 256 and >256 μg/ml, with an acceptable range of 16 to 64 μg/ml. For *C. perfringens*, agar dilution MICs were 2 to 4 dilutions higher than the E-test MICs; however, there were single colonies present within the ellipse that were isolated and retested. The repeat E tests on these colonies exhibited MICs that were within 1 dilution of the agar dilution MICs. *P. magnus* did not grow on WCA after overnight incubation, and MICs for *P. magnus* at 48 h were 1 to 2 dilutions lower than those measured on BBA by the agar dilution and E-test MIC methods.

DISCUSSION

The E test offers a simple method for susceptibility testing of all types of anaerobic bacteria. It is applicable for testing single isolates in those instances in which this information is of vital importance for selecting the most appropriate therapy. Results for rapidly growing organisms, such as the *B. fragilis* group and clostridia, are available after overnight incubation. Eighty-six percent of E-test MICs determined after overnight incubation were within 1 twofold dilution of the agar dilution MIC when BBA was used.

There was a tendency for the E-test MICs to be slightly lower than those measured by the agar dilution method, especially with imipenem, metronidazole, and to a lesser degree, penicillin. This could be explained in part by a difference in the inoculum that is actually delivered to the plate. Spots of inoculum delivered by the Steers replicator are approximately 5 mm in diameter and have an area of about 2 mm^2; thus, the final inoculum is approximately 5×10^4 CFU/mm^2. One-tenth of a milliliter of this same inoculum spread over a 15-cm-diameter plate results in a final inoculum of approximately 6×10^3 CFU/mm^2, which is almost a full log_{10} unit lower. Inoculum effects are variable depending on the antibiotic, the organism, and the agar base used for testing. In a study that compared the effects of inoculum and medium on MICs for *B. fragilis* group strains, Wexler et al. (20) reported that imipenem geometric mean MICs for the *B. fragilis* group were half of a twofold dilution lower from an inoculum of 10^5 to 10^4 organisms per spot when BBA was used. This is similar to the difference observed in our study. Although an inoculum of 10^8 CFU/ml has been used for disk diffusion tests with anaerobes (3, 9,

18), the use of an inoculum of 10^9 CFU/ml might yield E-test MICs more comparable to the agar dilution MICs for some of these agents and deserves further investigation.

A difference in the cefoxitin MICs for the *B. fragilis* group obtained by the two methods was evident on WCA. Agar dilution MICs for 9 of 38 of these strains were 16 to 32 μg/ml and E-test MICs were 128 to >256 μg/ml after overnight incubation. It is unlikely that the organisms degraded the cefoxitin in the plates, since a very small proportion of strains possess cefoxitin-degrading enzymes and cefoxitin is relatively stable to the β-lactamases present in *Bacteroides* spp. (19). This phenomenon also occurred with five strains of non-β-lactamase-producing *Clostridium* spp. and one strain of *Eubacterium* for which cefoxitin MICs were 32 to 64 μg/ml, as measured by agar dilution and E-test MICs that were consistently 1 to 2 twofold dilutions higher. Strains for which MICs were lower than 8 μg/ml did not exhibit this tendency. Because this did not occur on BBA to the same extent, we postulate that WCA contains an ingredient that destabilizes the antibiotic gradient for cefoxitin at the higher concentration.

MIC determinations for fusobacteria with beta-lactam agents yielded interesting results. Agar dilution tests typically produce tailing endpoints which make accurate MIC determinations difficult. Johnson et al. (11) studied this phenomenon and found the hazes to consist of L forms, and they concluded that since there is no convincing evidence that L forms have any clinical significance, the hazy growth should be ignored when reading agar dilution MIC plates. Our group (5) studied the effect of different agar bases on the hazing and found that different agars influence the extent of tailing endpoints. Regardless of the base used, the reading of agar dilution MIC endpoints is subject to more variability and inconsistency for this group of organisms than it is for other anaerobes. The E-test MICs were comparatively easy to read. There was a much clearer demarcation between the haze and the real growth, and thus, an accurate endpoint could be determined. While the agreement between the methods was within 1 twofold dilution in most instances, there was again a trend for the E test to yield slightly lower MICs. One major discrepancy occurred with a β-lactamase-producing strain of *F. nucleatum* and penicillin. The reason for this discrepancy is unclear, but it might be related to differences in growth rate between both methods, the rate of β-lactamase production, or the diffusion of penicillin from the E-test strip. The other β-lactamase-nonproducing *F. nucleatum* strain and two strains of *F. necrophorum* were exquisitely susceptible to penicillin. MICs for these organisms were <0.015 μg/ml by both methods. Because the current NCCLS document (16) lists the breakpoint for penicillin as 4 U/ml (or 2 μg/ml), we suggest that laboratories perform β-lactamase testing on *Fusobacterium* isolates for which penicillin MICs are >0.25 μg/ml. Additional tests with more β-lactamase-producing strains would be of interest.

The clindamycin MICs obtained by both methods after overnight incubation were 2 to 3 doubling dilutions lower than those observed after 48 h of incubation for many of the *B. fragilis* group strains. Thus, when the overnight E-test MICs were compared with agar dilution MICs obtained after 48 h of incubation, several discrepancies occurred. Barry et al. (3) evaluated a 24-h disk diffusion method for susceptibility testing of the *B. fragilis* group and modified the reference agar dilution method by reading the results at 24 h to make it more comparable to the disk diffusion technique for all antibiotics in the study. Overnight agar dilution MIC determinations in our study were more comparable to overnight E-test MICs than agar dilution MICs determined after the standard 48 h of incubation were.

Broth microdilution is the only method available for testing individual anaerobes. Panels for susceptibility testing of anaerobes are commercially available, but many do not have the selection of antimicrobial agents that are relevant for the individual patient. Although the broth microdilution method is considered an acceptable method for anaerobes, Aldridge and Sanders (1) reported that for 72% of the *B. fragilis* group isolates tested by broth microdilution, ceftizoxime MICs were ≥fourfold lower than the MICs obtained by agar dilution. Significant variation also occurred with cefoxitin and clindamycin. In addition, quality control MICs in the acceptable range for the reference strains have not been established for microdilution techniques (16).

Brook (6) reported that ceftizoxime MICs obtained by agar dilution correlated with efficacy in the eradication of *B. fragilis* and *B. thetaiotaomicron* from subcutaneous abscesses in a mouse model, while the MICs obtained by broth microdilution did not. Additionally, Lorian (15) has suggested that organisms growing on agar more closely simulate their state in the body. Therefore, agar methods may be more clinically relevant than broth methods. Additional studies to establish clinical correlation are needed.

The E test provides a simple and rapid method for quantitative susceptibility testing that is suitable for all anaerobes. Antibiotics can be selected for the individual patient. Fastidious organisms can be inoculated onto supplemented BBA, which supports the growth of almost all anaerobes. After application of the antibiotic carriers, incubation time is dependent on the rate of growth of the organism, and in many instances, MICs are available after 20 h. E-test MICs are generally in very good agreement with those obtained by the agar dilution method. BBA produced the fewest discrepancies with all the strains, with 97.8% categorical agreement, while supporting optimum growth. WCA was not suitable for testing cefoxitin with the *B. fragilis* group. The E test is a reliable overnight method for susceptibility testing of rapidly growing anaerobic bacteria. Slower-growing strains yield good results after 48 h of incubation.

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