First Comprehensive Evaluation of the M.I.C. Evaluator Device Compared to Etest and CLSI Reference Dilution Methods for Antimicrobial Susceptibility Testing of Clinical Strains of Anaerobes and Other Fastidious Bacterial Species

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The new M.I.C. Evaluator strip uses test methodology and the recording of results that are similar to those of Etest. For this first assessment, 102 clinical strains of anaerobic bacteria from 12 genera and 155 strains from 7 genera and 8 species of fastidious bacteria were tested by M.I.C. Evaluator, Etest, and agar dilution or broth microdilution as a reference standard. Ampicillin, amoxicillin, amoxicillin-clavulanate, cefotaxime, ciprofloxacin, erythromycin, imipenem, levofloxacin, metronidazole, penicillin, and tetracycline were tested depending on the species. Agar dilution for anaerobes was performed according to CLSI document M11-A7. For the fastidious bacteria, CLSI document M45-A2 was followed. For the anaerobes, essential and categorical agreement between M.I.C. Evaluator and Etest was >90%. Compared to agar dilution, essential agreement was low for both strip tests, and many very major errors were observed for metronidazole (13 to 14%) and penicillin (8 to 9%) with isolates from the *Bacteroides fragilis* group and *Clostridium* species. For fastidious species, essential agreements for M.I.C. Evaluator and Etest plus or minus one doubling dilution were >95%. Compared to broth microdilution, essential agreements were low (40 to 90%) plus or minus one dilution and were >90% plus or minus two dilutions, with high overall category agreement (CA). Major and minor errors were within established parameters for all strains tested. The M.I.C. Evaluator strips were equivalent to Etest for anaerobes and fastidious species. These observations require further investigation to determine which methods provide the most accurate MIC for clinical utility. The further evaluation of additional M.I.C. Evaluator agents will be performed as they become available.

Full identification and antimicrobial susceptibility testing of both anaerobes and fastidious microorganisms is usually reserved for cases where an isolate is either in pure culture or is the predominant pathogen. In cases of sepsis, extragenital infections, brain abscess, subdural abscess, or deep wounds, it is important to discover the presence of these species and to investigate their susceptibility to effective antimicrobial agents (2, 3, 13, 15, 20, 22). However, the testing of susceptibilities for anaerobic and fastidious bacteria is hampered by slow growth, variations in media that support the growth of these microorganisms, and test methodologies.

For anaerobes, there have been many systems used for determining the susceptibility profiles of clinical isolates (13, 15, 25, 26). Most are time-consuming and are not amenable to routine diagnostic clinical laboratories. The Clinical and Laboratory Standards Institute has published a standard agar dilution method for testing anaerobes against a variety of agents (9). One of the difficulties encountered with the susceptibility testing of anaerobes using endpoint agar or broth dilution tests has been with reading and interpreting the endpoints. The CLSI document M11-A7 also contains a pictorial guide, developed from the consensus of experts, that provides some assistance to laboratories on how to read growth endpoints for agar dilution. These are meant as a guide and help different persons read the results in different laboratories to get essentially similar MICs for a particular drug-microorganism combination.

While the CLSI agar dilution method provides reproducible results, the method is not conducive for the susceptibility testing

of an individual isolate recovered from a serious anaerobic infection; rather, it is useful for surveillance testing to monitor changing resistance patterns among anaerobes in various populations (7, 16, 17). Other methods, including disk elution or broth dilution methods in prerduced media, have not provided reproducible results (16). The most commonly used method in routine laboratories is now the gradient endpoint method (5, 6, 12, 27, 28). It provides rapid and accurate MICs for therapeutic options of the most common antimicrobial agents that are effective against anaerobes, and until now the Etest device was the only gradient endpoint method available.

The antimicrobial susceptibility testing of fastidious bacterial species also has been difficult due to differing growth and media requirements, the specific atmospheres required for growth, and the fact that some of these fastidious species grow poorly in broth culture. Many of these microorganisms most often are tested by disk diffusion or agar dilution on a variety of media (11). Like anaerobic bacteria, these microorganisms are amenable to testing by gradient endpoint susceptibility, and Etest is often used in clin-

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TABLE 1 Reference method conditions and antimicrobial agents tested for seven fastidious bacterial genera

Organism	Test medium	Incubation conditions	Atmosphere	Antimicrobial(s) tested ^a
<i>N. gonorrhoeae</i>	GC agar base with 1% defined supplement	35°C; 20–24 h	5% CO ₂	AMP, CTX, CIP, PEN, TET
<i>N. meningitidis</i>	Mueller-Hinton agar with 5% sheep blood	35°C; 18–20 h	5% CO ₂	AMP, CTX, CIP, PEN, TET
<i>Haemophilus</i> species	Supplemented <i>Haemophilus</i> test medium agar	35°C; 18–20 h	5% CO ₂	AMP, AMX, AMC, CTX, CIP, ERY, IMP, LEV, TET
<i>Campylobacter</i> species	Mueller-Hinton agar with 5% sheep blood	42°C; 24 h	Microaerophilic	CIP, ERY
<i>L. monocytogenes</i>	Mueller-Hinton agar with 5% sheep blood	35°C; 18–24 h	Ambient air	AMP, ERY
<i>P. multocida</i>	Mueller-Hinton agar with 5% sheep blood	35°C; 18–20 h	5% CO ₂	PEN
<i>M. catarrhalis</i>	Mueller-Hinton agar with 5% sheep blood	35°C; 18–20 h	Ambient air	AMX

^a AMP, ampicillin; AMX, amoxicillin; AMC, amoxicillin-clavulanate; CTX, cefotaxime; CIP, ciprofloxacin; ERY, erythromycin; IMP, imipenem; LEV, levofloxacin; PEN, penicillin; TET, tetracycline.

ical laboratories (4, 8, 14). The importance of accurate testing for monitoring the antimicrobial resistance of the microorganisms has been identified in many instances (18, 19, 23, 29).

A new gradient endpoint device, the M.I.C. Evaluator (M.I.C.E), produced by Thermo Fisher Scientific (Basingstoke, United Kingdom), is now available for the MIC testing of a variety of bacterial species. The M.I.C. Evaluator strip operates on a principle similar to that of the Etest device. There have been only two publications comparing M.I.C. Evaluator strips to the Etest device. One compared the strip devices to the British Society for Antimicrobial Chemotherapy (BSAC) method (21), and the other comparison was only for beta-lactamase-producing *Haemophilus influenzae* (28).

The present study was designed to validate the M.I.C. Evaluator device for testing a panel of recently isolated anaerobic bacteria recovered from serious anaerobic infections and a collection of clinical strains of a number of commonly recovered fastidious species. These strains were all isolated from patients in our large quaternary care facility to provide an in-use validation of the technologies. The M.I.C. Evaluator and Etest devices were compared for accuracy and reproducibility according to the standard CLSI agar dilution MIC methods for these microorganisms. Other collections of aerobic and facultative anaerobic Gram-positive and Gram-negative bacterial species are reported in a companion publication (24).

MATERIALS AND METHODS

Bacterial strains. (i) **Anaerobes.** A total of 102 anaerobic strains, each from an individual patient, were tested by agar dilution according to CLSI methodology (9), by Etest, and with the M.I.C. Evaluator device. There were 12 common genera and 28 species of anaerobes in this collection of strains. The species were *Bacteroides fragilis* (21 strains), *B. caccae* (1 strain), *B. vulgatus* (1 strain), *Bacteroides* species (1 strain), *Clostridium perfringens* (7 strains), *C. ramosum* (3 strains), *C. clostridioforme* (1 strain), *C. septicum* (2 strains), *C. tertium* (1 strain), *C. difficile* (3 strains), *Clostridium* species other than *C. perfringens* (5 strains), *Finegoldia magna* (13 strains), *Peptostreptococcus anaerobius* (2 strains), *Peptoniphilus harei* (1 strain), *Propionibacterium acnes* (8 strains), *Prevotella bivia* (1 strain), *Prevotella* species (3 strains), *Eggerthella lenta* (6 strains), *Fusobacterium nucleatum* (5 strains), *F. necrophorum* (2 strains), *F. mortiforum* (1 strain), *Fusobacterium* species (1 strain), *Veillonella* species (1 strain), *Actinomyces meyeri* (5 strains), *Actinomyces* species (1 strain), and anaerobic Gram-positive cocci (6 strains). Two standard quality control strains were tested. These were *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741.

The strains were identified by standard identification methods for anaerobic bacteria. This included Gram stain morphology, differential disk tests, prereduced anaerobically sterilized (PRAS) identification tests, and gas-liquid chromatography. A small number of anaerobic Gram-pos-

itive cocci could not be identified to the genus level but most likely were *Peptococcus* species, as this is the only genus of anaerobic staphylococcal-like microorganisms.

(ii) **Fastidious bacterial species.** A total of 155 recent, clinical strains of fastidious bacterial species were recovered from separate patients at the University of Alberta Hospital/Stollery Children's Hospital, Edmonton, Alberta, Canada. Organisms from the following genera were tested: 49 *Haemophilus* species (39 *H. influenzae* and 10 *H. parainfluenzae*), 49 *Neisseria* species (27 *N. gonorrhoeae* and 22 *N. meningitidis*), 19 *Campylobacter jejuni* and *C. coli*, 9 *Pasteurella multocida*, 20 *Listeria monocytogenes*, and 10 *Moraxella catarrhalis*. Quality control strains of *Streptococcus pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, and *N. gonorrhoeae* ATCC 49226 were tested when broth microdilution trays and agar dilution plates were prepared and with each testing period.

Antimicrobial agents. For anaerobes, four agents were tested: amoxicillin-clavulanate, imipenem, metronidazole, and penicillin. The concentration ranges were similar for Etest and the M.I.C. Evaluator device. M.I.C. Evaluator strips were provided by Thermo Fisher Scientific, Basingstoke, United Kingdom. Etest strips were purchased from the manufacturer (AB Biodisk-bioMérieux). For the fastidious species, the antimicrobial agents tested for each species are described in Table 3. These included ampicillin, amoxicillin, amoxicillin-clavulanate, cefotaxime, ciprofloxacin, erythromycin, imipenem, levofloxacin, penicillin, and tetracycline.

Antimicrobial susceptibility testing of anaerobes. Agar dilution and endpoint tests were performed using the same inoculum for each test. Strains were grown on anaerobic blood agar plates for 48 h at 35°C. Representative colonies were picked to sterile saline to a density equivalent to a 1 McFarland standard. This inoculum then was used to prepare the wells for agar dilution and for the gradient endpoint tests. Agar dilution tests were performed on Brucella agar (Oxoid, Basingstoke, United Kingdom) supplemented with 5% laked sheep blood, hemin, and vitamin K according to CLSI guidelines. The growth endpoints for agar dilution were determined visually with the aid of the photographic charts supplied in CLSI document M11-A7 (9), and breakpoints were recorded according to information in the most recent supplement (10).

M.I.C. Evaluator and Etest strips were tested in a similar manner according to the manufacturers' instructions. After the inoculation of the plates, strips for each agent were added to the plates according to the manufacturers' instructions and the plates were incubated for 24 to 72 h at 35°C in an anaerobic atmosphere. Inhibition for each strip was read at the point where the elliptical zone intersected with the strip.

Antimicrobial susceptibility testing of fastidious species. Agar dilution and gradient endpoint tests were performed using the same inoculum. Standard CLSI broth methodologies (11) of broth microdilution and agar dilution (for *N. gonorrhoeae*) (Table 1) were used as the reference standards to test susceptibility. M.I.C. Evaluator and Etest strips of the appropriate antimicrobials listed in Table 1 were added. Inhibition was read for each manufacturer's strip at the point where the elliptical zone intersected with the strip.

TABLE 2 Number (%) of categorical errors for M.I.C.E and Etest compared to agar dilution for 102 clinical strains of anaerobic bacteria

Antimicrobial agent	No. of strains in each resistance category			No. (%) of errors						% Agreement (CA/EA ^a)	
	S	I	R	Minor		Major		Very major		M.I.C.E	Etest
				M.I.C.E	Etest	M.I.C.E	Etest	M.I.C.E	Etest		
Amoxicillin-clavulanate	92	3	7	1 (1)	7 (7)	1 (1)	0 (0)	1 (1)	1 (1)	97/74	92/50
Imipenem	92	3	7	2 (2)	4 (4)	0 (0)	0 (0)	3 (3)	4 (4)	95/49	92/44
Metronidazole	55	0	47	0 (0)	1 (1)	0 (0)	0 (0)	14 (14)	13 (13)	86/63	86/56
Penicillin	50	1	51	8 (8)	8 (8)	1 (1)	1 (1)	8 (8)	9 (9)	83/74	82/72
Total				11 (2.7)	20 (5)	2 (0.4)	1 (0.2)	26 (6.4)	27 (6.6)	90/66	88/59

^a CA/EA, categorical and essential agreement defined by comparison to agar dilution.

Analysis. All MICs were interpreted using CLSI clinical breakpoints. The comparison of both endpoint methods to the reference method was done using categorical and essential agreement, a method commonly used to compare different antimicrobial susceptibility testing (AST) methods. Categorical agreement is based on interpretive breakpoints of sensitive (S), intermediate (I), and resistant (R). A very major error is defined as the reference result being resistant and the test result being susceptible. A major error is defined as the reference result being susceptible and the test result being resistant. A minor error is defined as the reference result being resistant or susceptible and the test result being intermediate, or the reference result being intermediate and the test result being susceptible or resistant.

Essential agreement is based on the number of MICs plus or minus one doubling dilution of the reference MIC. The FDA requires >90% essential and category agreement, <1.5% very major errors, <3% major errors, and <10% minor errors.

RESULTS

The susceptibilities of the two quality control strains for anaerobes and the three quality control strains for the fastidious species were tested at the time of production of broth microdilution trays, agar dilution, or gradient endpoint dilution tests and each time a batch of clinical strains were examined. All quality control strains were tested at least three times during the clinical isolate test period. All quality control strains were within acceptable ranges for all antimicrobials tested and were highly reproducible. For the anaerobes, results from the antimicrobials tested were not affected by the method of reading, either reading an elliptical zone by M.I.C.E or Etest or by the visual interpretation of growth on the agar dilution plates.

Very major, major, and minor error rates and the categorical

and essential agreements for M.I.C. Evaluator and Etest compared to agar dilution for 102 clinical strains of anaerobic bacteria tested against four primary anaerobic agents are shown in Table 2. Agar dilution MICs were generally greater for all species and antimicrobial agents tested. For penicillin and metronidazole, this resulted in more very major errors for both M.I.C. Evaluator and Etest. The numbers of major and minor errors were low. Essential agreement for all four agents tested was also low. Only amoxicillin-clavulanate and imipenem had categorical agreement of greater than 90% compared to that of agar dilution. The overall agreements for both the M.I.C. Evaluator and Etest strips were 90 and 88% for the 408 drug-microorganism combinations.

While this study was designed to compare the two gradient endpoint devices to the reference method, it was possible to also compare the two gradient endpoint devices to each other. For most of the anaerobes, the M.I.C. Evaluator strip result was either the same as or one-half to one doubling dilution higher than that for the Etest device. Agreement between the M.I.C. Evaluator strips and Etest for all agents tested was greater than 90% (93 to 99%). Essential agreement was also excellent (96 to 100%) between these two methods, except for amoxicillin-clavulanate (83%), although this was greater than 90% at plus or minus two doubling dilutions. The differences with amoxicillin-clavulanate were mainly in the *Bacteroides fragilis* group strains, but there were only two categorical errors for this group of microorganisms.

For the fastidious bacterial species, the essential agreements at plus or minus one doubling dilution compared to reference methods were lower (Table 3), but the categorical agreements were excellent. All were greater than 90%, except for penicillin and

TABLE 3 Essential and categorical agreement for 155 fastidious bacterial strains tested by M.I.C.E and Etest compared to the reference method

Antimicrobial agent	No. of strains tested	Essential agreement (%)		Categorical agreement ^a (%)	
		M.I.C.E	Etest	M.I.C.E	Etest
Ampicillin	117	47/117 (40)	59/117 (50)	83/90 (92)	83/90 (92)
Amoxicillin	49	37/49 (76)	39/49 (80)	NA ^a	NA ^a
Amoxicillin-clavulanate	59	24/59 (41)	34/59 (58)	59/59 (100)	59/59 (100)
Cefotaxime	97	74/97 (76)	76/97 (78)	97/97 (100)	97/97 (100)
Ciprofloxacin	113	81/113 (72)	86/113 (76)	111/113 (98)	111/113 (98)
Erythromycin	85	54/85 (64)	67/85 (79)	16/16 ^b (100)	16/16 ^b (100)
Imipenem	49	30/49 (61)	29/49 (59)	49/49 (100)	48/49 (98)
Levofloxacin	49	40/49 (82)	42/49 (86)	49/49 (100)	49/49 (100)
Penicillin	57	35/57 (61)	37/57 (65)	50/57 (88)	52/57 (91)
Tetracycline	97	86/97 (89)	83/97 (86)	89/97 (92)	87/97 (90)

^a NA, CLSI breakpoints not available for these tests.

^b Breakpoints were not available for all species tested against erythromycin.

TABLE 4 Minor and major error rates for 155 fastidious bacterial strains tested by M.I.C.E and Etest compared to the reference method

Antimicrobial agent and error type	Error rate for:			
	M.I.C. Evaluator		Etest	
	No. (%) of errors	Species (no.) with errors	No. (%) of errors	Species (no.) with errors
Minor				
Ampicillin	3/87 (3)	<i>N. meningitidis</i> (2) <i>Haemophilus</i> spp. (1)	4/87 (5)	<i>N. meningitidis</i> (2) <i>Haemophilus</i> spp. (2)
Ciprofloxacin	1/110 (<1)	<i>Campylobacter</i> spp.	1/110 (<1)	<i>Campylobacter</i> spp.
Penicillin	7/57 (12)	<i>N. meningitidis</i> (2) <i>N. gonorrhoeae</i> (5)	5/57 (9)	<i>N. meningitidis</i> (1) <i>N. gonorrhoeae</i> (4)
Tetracycline	7/94 (7)	<i>Haemophilus</i> spp. (1) <i>N. meningitidis</i> (3) <i>N. gonorrhoeae</i> (3)	11/94 (12)	<i>Haemophilus</i> spp. (1) <i>N. meningitidis</i> (5) <i>N. gonorrhoeae</i> (5)
Major				
Ampicillin	4/87 (5)	<i>Haemophilus</i> spp. (4)	3/87 (3)	<i>Haemophilus</i> spp. (3)
Ciprofloxacin	1/110 (<1)	<i>Campylobacter</i> spp. (1)	1/110 (<1)	<i>Campylobacter</i> spp. (1)
Imipenem	0		1/46 (2)	<i>Haemophilus</i> spp. (1)
Tetracycline	1/94 (1)	<i>Haemophilus</i> spp. (1)	0	

tetracycline (88%). As shown in Table 3, the essential agreement for M.I.C. Evaluator compared to Etest for all agents tested, except imipenem and tetracycline, was lower. When the essential agreement limit was not met, the M.I.C. Evaluator result usually was greater than the Etest result. At plus or minus two doubling dilutions, the essential agreements were greater than 85% for all drug and microorganism combinations. As shown in Table 4, there were no very major errors compared to the reference methods. The minor errors were mostly found with penicillin and tetracycline, while the major errors were mainly with ampicillin and *Haemophilus* species (i.e., they were more resistant by M.I.C. Evaluator and Etest). The percentages of errors were well within acceptable ranges, and there was no significant difference when the two gradient endpoint methods were compared to each other.

DISCUSSION

Most clinical microbiology laboratories perform relatively small numbers of antimicrobial susceptibility tests for anaerobes and fastidious bacterial species. For anaerobes, the CLSI reference agar dilution method requires a significant outlay in time, culture media, methodology, and expertise to be performed effectively. Similarly, as shown in Table 1, several different media and culture methods are required to encompass susceptibility testing for almost all of the fastidious species. Furthermore, most of the work that has been done in the ongoing development of these methods has been in the quality control area. For anaerobic agents where the MICs are low, the correlation between agar dilution and Etest has been excellent (6).

The gradient endpoint methods, particularly Etest and now the M.I.C. Evaluator strips, offer an easier, more readily available alternative to either agar dilution or in-house-prepared broth microdilution for the daily susceptibility testing of anaerobes and fastidious microorganisms isolated from important clinical infections where these species can thrive.

There have been few reported studies that have compared these methods directly (1, 12, 21). In the current study, with the first four primary anti-anaerobic agents our goal was to compare results of the M.I.C. Evaluator and Etest strips to reference MIC results.

For the anaerobes, the differences between the gradient endpoint methods and agar dilution, especially for metronidazole and penicillin, are of interest. Both agents showed excellent reproducibility both in the quality control results and when clinical strains were compared between the M.I.C. Evaluator and Etest strips. The very major errors were observed among *Bacteroides fragilis* group strains and *Clostridium* species, both *C. perfringens* and other clostridia. The same phenomenon was observed for penicillin. The reasons for these discordant results are not well understood. When tests were repeated the same observations were made, so it was not due to reading or recording errors. Not all strains within a species showed these differences. It is more puzzling since the same inoculum was utilized for all three tests, and they were performed at the same time.

These observations suggest that the agar dilution method is overcalling resistance in some strains of anaerobic bacteria with certain agents. For antifungal susceptibility testing, the opposite has been observed. It has been shown that amphotericin B resistance may not be recognized as readily with the standard reference method as with Etest (30). In the current study, either resistance is not recognized with the gradient endpoint methods or the agar dilution method is overcalling the MIC of the strain. It will be important to work out such differences to ensure that clinical outcomes are not affected by an *in vitro* laboratory result performed by one or the other method. It should be noted, however, that the mechanisms of deposition of the antimicrobial agent even in an agar medium are not the same for agar dilution and the gradient endpoint methods. Therefore, it might be expected that different results can be obtained with some strains or species depending on the method of MIC analysis.

At least for the gradient endpoint devices, our observations showed that the M.I.C. Evaluator strips for the anaerobic agents performed equivalently to the Etest predicate device. Quality control results were all within range, and essential and categorical agreements were within what would be considered excellent parameters. The categorical errors were a few additional minor errors for Etest for *Bacteroides fragilis* and amoxicillin-clavulanate.

Observations for the fastidious species tested showed that cat-

egorical agreements of M.I.C. Evaluator strips and Etest were essentially equivalent and were highly comparable to the various reference methods used for each group of organisms. Lower essential agreements (at plus or minus one doubling dilution) were observed, which were found to be much higher at plus or minus two doubling dilutions. Strains of these species have very low MICs for both quality control and clinical isolates, with some being as low as 0.002 mg/liter. Therefore, a 2-fold difference (e.g., 0.004 to 0.012 mg/liter) has no effect on categorical differences and may be only a reading issue with some strains. Such differences at these low MICs are not considered significant.

The observation of no very major errors, either between M.I.C. Evaluator and Etest strips or between these gradient endpoint methods and the reference methods, indicates that the gradient endpoint methods can be used for the antimicrobial susceptibility testing of this varied group of fastidious bacterial species.

The equivalence of the M.I.C. Evaluator strips and Etest method in this study suggests that both methods can be used to provide reproducible and accurate MICs for the reporting of anaerobic and fastidious bacterial susceptibilities. Further studies on other agents being developed for the M.I.C. Evaluator strips will permit an expansion of the capabilities of this test for the routine antimicrobial susceptibility testing of these microorganisms. The M.I.C. Evaluator strips are also available in single test pouches, which will increase the capability for their use in smaller clinical laboratories that have a need for on-site susceptibility testing.

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REFERENCES

- Abdel-Rahman EM, Ismael NA, Dixon RA. 2000. Comparison of Etest and agar dilution methods of susceptibility testing of resistant isolates of *Haemophilus influenzae* from Saudi Arabia. *J. Antimicrob. Chemother.* 46:148–149.
- Albertson TE, Louie S, Chan AL. 2010. The diagnosis and treatment of elderly patients with acute exacerbation of chronic obstructive pulmonary disease and chronic bronchitis. *J. Am. Geriatr. Soc.* 58:570–579.
- Allerberger F, Wagner M. 2010. Listeriosis: a resurgent foodborne infection. *Clin. Microbiol. Infect.* 16:16–23.
- Billal DS, Hotomi M, Yamanaka N. 2007. Can the Etest correctly determine the MICs of β -lactam and cephalosporin antibiotics for beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae*? *Antimicrob. Agents Chemother.* 51:3463–3464.
- Bolmström A. 1993. Susceptibility testing of anaerobes with Etest. *Clin. Infect. Dis.* 16(Suppl. 4):S367–S370.
- Bolmström A, et al. 2007. Validation and reproducibility assessment of tigecycline MIC determinations by Etest. *J. Clin. Microbiol.* 45:2474–2479.
- Brazier JS, Hall V, Morris TE, Gal M, Duerden BI. 2003. Antibiotic susceptibilities of Gram-positive anaerobic cocci: results of a sentinel study in England and Wales. *J. Antimicrob. Chemother.* 52:224–228.
- Chisholm SA, et al. 2010. Cephalosporin MIC creep among gonococci: time for a pharmacodynamic rethink? *J. Antimicrob. Chemother.* 64: 2141–2148.
- Clinical and Laboratory Standards Institute. 2007. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard, 7th ed., vol. 27, no. 2. M11-A7. CLSI, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing of anaerobic bacteria; informational supplement. M11-S1. CLSI, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2010. Methods of antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline, 2nd ed. M45-A2. CLSI, Wayne, PA.
- Croco JL, Erwin MEJ, Jennings ML, Putman R, Jones RN. 1994. Evaluation of the Etest for antimicrobial spectrum and potency determinations of anaerobes associated with bacterial vaginosis and peritonitis. *Diagn. Microbiol. Infect. Dis.* 20:213–219.
- Finegold SM. 1997. Perspective on susceptibility testing of anaerobic bacteria. *Clin. Infect. Dis.* 25(Suppl. 2):S251–S253.
- Fuchs PC, Barry AL, Brown SD. 2001. Influence of variations in test methods on susceptibility of *Haemophilus influenzae* to ampicillin, azithromycin, clarithromycin and telithromycin. *J. Clin. Microbiol.* 39:43–46.
- Goldstein EJ, Citron DMP, Goldman J, Goldman RJ. 2008. National hospital survey of anaerobic culture and susceptibility methods: III. *Anaerobe* 14:68–72.
- Goldstein EJC, Citron DM. 2011. Resistance trends in antimicrobial susceptibility of anaerobic bacteria, part I. *Clin. Microbiol. Newslett.* 33: 1–8.
- Goldstein EJC, Citron DM. 2011. Resistance trends in antimicrobial susceptibility of anaerobic bacteria, part II. *Clin. Microbiol. Newslett.* 33: 9–15.
- Knapp JS, et al. 1997. Antimicrobial susceptibilities of strains of *Neisseria gonorrhoeae* in Bangkok, Thailand: 1994–1995. *Sex. Transm. Dis.* 24:142–148.
- Knapp JS, Fox KK, Trees DL, Whittington WL. 1997. Fluoroquinolone resistance in *Neisseria gonorrhoeae*. *Emerg. Infect. Dis.* 3:33–39.
- Morse SA. 1996. *Neisseria, Moraxella, Kingella, and Eikenella*. In Baron S (ed.), *Medical microbiology*, 4th ed. University of Texas Medical Branch, Galveston TX.
- Mushtaq S, Warner M, Cloke J, Mariya A-S, Livermore DM. 2010. Performance of the Oxoid M.I.C. Evaluator strips compared with the Etest assay and BSAC agar dilution. *J. Antimicrob. Chemother.* 65:1702–1711.
- Needham CA. 1988. *Haemophilus influenzae*: antibiotic susceptibility. *Clin. Microbiol. Rev.* 1:218–227.
- Pfaller MA, Jones RN. 2006. Performance accuracy of antibacterial and antifungal susceptibility test methods. Report from the College of American Pathologists Microbiology Surveys Program (2001–2003). *Arch. Pathol. Lab. Med.* 130:767–778.
- Rennie RP, Turnbull L, Brosnikoff C. 2011. First comprehensive evaluation of the M.I.C. Evaluator device compared to Etest and CLSI broth microdilution for MIC testing of aerobic Gram-positive and Gram-negative bacterial species. *J. Clin. Microbiol.* 50:1147–1152.
- Rosenblatt JE. 1984. Antimicrobial susceptibility testing of anaerobic bacteria. *Rev. Infect. Dis.* 6(Suppl. 1):S242–S248.
- Rosenblatt JE. 1989. Susceptibility testing of anaerobic bacteria. *Clin. Lab. Med.* 9:239–254.
- Rosenblatt JE, Gustafson DR. 1995. Evaluation of the Etest for susceptibility testing of anaerobic bacteria. *Diagn. Microbiol. Infect. Dis.* 22:279–284.
- Schieven BC, Massey VE, Lannigan R, Hussain Z. 1995. Evaluation of susceptibility of anaerobic organisms by the Etest and the reference agar dilution method. *Clin. Infect. Dis.* 20(Suppl. 2):S337–S338.
- Tristram SG. 2008. A comparison of Etest, M.I.C. Evaluator strips and CLSI broth microdilution for determining β -lactam antimicrobial susceptibility in *Haemophilus influenzae*. *J. Antimicrob. Chemother.* 62: 1464–1466.
- Wanger A, Mills K, Nelson PW, Rex JH. 1995. Comparison of Etest and National Committee for Clinical Laboratory Standards broth microdilution method for antifungal susceptibility testing: enhanced ability to detect amphotericin B-resistant *Candida* isolates. *Antimicrob. Agents Chemother.* 39:2520–2522.