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Quantitative Antimicrobial Susceptibility Testing of *Haemophilus influenzae* and *Streptococcus pneumoniae* by Using the E-Test

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The E-test (PDM Epsilometer; AB Biodisk, Solna, Sweden) is an antimicrobial agent gradient-coated plastic test strip which allows MIC determinations on agar media. The test is performed in a manner similar to the agar disk diffusion procedure. A collection of *Haemophilus influenzae* and *Streptococcus pneumoniae* strains possessing various resistance mechanisms was used to evaluate the E-test method. *H. influenzae* strains were tested with both *Haemophilus* test medium (HTM) and PDM ASM II chocolate agar, while the *S. pneumoniae* strains were tested on Mueller-Hinton sheep blood agar. E-test MICs for a total of 10 antimicrobial agents were compared with broth microdilution MICs determined according to National Committee for Clinical Laboratory Standards methods. In general, E-test MICs for both species were quickly and easily interpreted and agreed within one $\log_2$ MIC increment in 89.8% of tests with *H. influenzae* and in 80.4% of pneumococcal tests. The majority of disagreements between the E-test and conventional MICs occurred with trimethoprim-sulfamethoxazole because of trailing and diffuse E-test MIC endpoints with both species. Ampicillin MICs for β-lactamase-producing *H. influenzae* determined by the E-test differed at times from those determined by conventional testing because of the vagaries of interpreting colonies growing within the E-test inhibition ellipses. E-test penicillin MICs for pneumococci tended to be 1 to 2 $\log_2$ dilutions lower than those determined by using Mueller-Hinton broth supplemented with lysed horse blood. Nevertheless, strains of both species with documented resistance to the study drugs were detected by E-tests, i.e., 0.7% of the tests had very major errors with *H. influenzae* and 0.8% had very major errors with *S. pneumoniae*. Thus, the E-test represents a potential alternative method for antimicrobial susceptibility testing of these two fastidious bacterial species.

The E-test (PDM Epsilometer; AB Biodisk, Solna, Sweden) is a new product for quantitative (MIC) antimicrobial susceptibility testing. The E-test consists of a plastic strip with a predefined antimicrobial agent concentration gradient immobilized on one side and a continuous MIC scale covering 15 twofold dilutions on the opposite side. To determine an MIC with the E-test, the surface of an agar plate is swab inoculated with an adjusted bacterial suspension in the same manner as a disk diffusion test. One or more E-test strips for the antimicrobial agents to be tested are then placed on the inoculated agar surface. After overnight incubation, the interaction of the antimicrobial agent gradient and the test bacterial inoculum gives rise to elliptical inhibitory zones. The intersection of the growth ellipse margin with the E-test strip gradient indicates the MIC of the drug for the organism.

Increasing resistance by various mechanisms to several classes of antimicrobial agents among clinical isolates of *Haemophilus influenzae* (3, 9, 11) and *Streptococcus pneumoniae* (7, 8) has focused new attention on reliable methods for in vitro susceptibility testing of these species and on guidelines for interpretation of such tests (2, 4–6, 10). The National Committee for Clinical Laboratory Standards (NCCLS) has recently endorsed new media, quality control guidelines, and interpretive criteria for *Haemophilus* testing (12, 13) and is exploring potential new standards for pneumococcal testing. The purpose of the present study was to evaluate the E-test for determining the susceptibilities of *H. influenzae* and *S. pneumoniae* to antimicrobial agents of clinical relevance for those species.

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MATERIALS AND METHODS

Antimicrobial agents. E-test strips containing ampicillin, penicillin, cefaclor, cefuroxime, cefotaxime, chloramphenicol, erythromycin, doxycycline, tetracycline, and trimethoprim-sulfamethoxazole were provided by the manufacturer for the purpose of this study. Reagent grade powders of the same antimicrobial agents were used for performance of reference agar and broth dilution MIC tests.

Test strains. One hundred strains of *H. influenzae* and 50 strains of *S. pneumoniae* which demonstrated various resistance mechanisms and levels of antimicrobial susceptibility (Table 1) were utilized for this evaluation.

Control strains. The control strains employed in this study included *H. influenzae* ATCC 49247 and ATCC 10211, *Escherichia coli* ATCC 25922, and *Streptococcus faecalis* (*Enterococcus faecalis*) ATCC 29212.

E-tests. E-tests of *H. influenzae* were performed on both *Haemophilus* test medium (HTM) agar (B-D Microbiology Systems [BDMS], Cockeysville, Md.) and PDM ASM II agar (Biodisk). The latter medium was supplemented with 1% hemoglobin (BDMS) and 1% IsoVitaleX (BDMS) and is hereafter referred to as ASM II chocolate agar. For *S. pneumoniae*, E-tests were performed on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (BDMS). Inocula were prepared by direct suspension in Mueller-Hinton broth of colonies grown overnight on enriched chocolate agar (*H. influenzae*) or sheep blood agar (pneumococci) to achieve turbidity equivalent to a 0.5 McFarland opacity standard. The 150-mm-diameter agar plates were inoculated by confluent swabbing of the surface with the adjusted inoculum suspensions. With both species, four or five E-test strips were placed in an equidistant radial fashion on the surface of the plates. After application of the E-test strips, *H. influenzae* plates were incubated at 35°C in

TABLE 1. Resistance properties[a] of 100 *H. influenzae* and 50 *S. pneumoniae* test strains

Species and resistance property	No. of strains with resistance property
H. influenzae	
β-Lactamase positive	53
β-Lactamase negative, ampicillin resistant (MIC ≥ 4 μg/ml)	5
Chloramphenicol resistant (CAT positive)[b]	10
Tetracycline resistant (MIC ≥ 16 μg/ml)	14
Trimethoprim-sulfamethoxazole resistant (MIC ≥ 4 μg/ml)	5
Fully susceptible	37
S. pneumoniae	
Penicillin resistant (MIC ≥ 2 μg/ml)	4
Penicillin relative resistance (MIC = 0.12 to 1 μg/ml)	18
Chloramphenicol resistant (CAT positive)[b]	5
Erythromycin resistant (MIC ≥ 8 μg/ml)	2
Tetracycline resistant (MIC ≥ 16 μg/ml)	14
Trimethoprim-sulfamethoxazole resistant (MIC ≥ 4 μg/ml)	19
Fully susceptible	3

[a] Some strains were multiply resistant.
[b] Produced chloramphenicol acetyltransferase (CAT).

5% CO_2 for 16 to 18 h, while pneumococcal test plates were incubated in ambient air at 35°C for 20 to 22 h. The longer incubation period for pneumococcal tests was chosen on the basis of preliminary studies that indicated better definition of growth ellipses after 20 to 22 h than after only 16 to 18 h of incubation. The use of CO_2 for E-tests of *H. influenzae* was based on prior experience, which showed that some strains do not grow well on HTM agar if incubated in ambient air (5). E-test MICs were interpreted by noting the point of intersection of the growth ellipse margin with the MIC scale on the E-test strip when viewed from the upper agar surface with the plate lids removed (see Fig. 1 and 2).

Reference broth microdilution susceptibility tests. Broth microdilution MIC tests were performed in the manner recommended by the NCCLS (12) by using HTM broth (BDMS) for *H. influenzae* and cation adjusted (25 mg of Ca^{2+} per liter and 12.5 mg of Mg^{2+} per liter) Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with 3% lysed horse blood for *S. pneumoniae* (12). With both species, the final inoculum density was ca. 5×10^5 CFU/ml, and incubation was carried out at 35°C in ambient air for 20 to 24 h.

Agar dilution tests. Agar dilution MIC tests were also performed with *H. influenzae* by using twofold concentration increments of the antimicrobial agents incorporated in molten ASM II chocolate agar in the manner suggested by the NCCLS for nonfastidious bacteria (12). Inoculum sus-

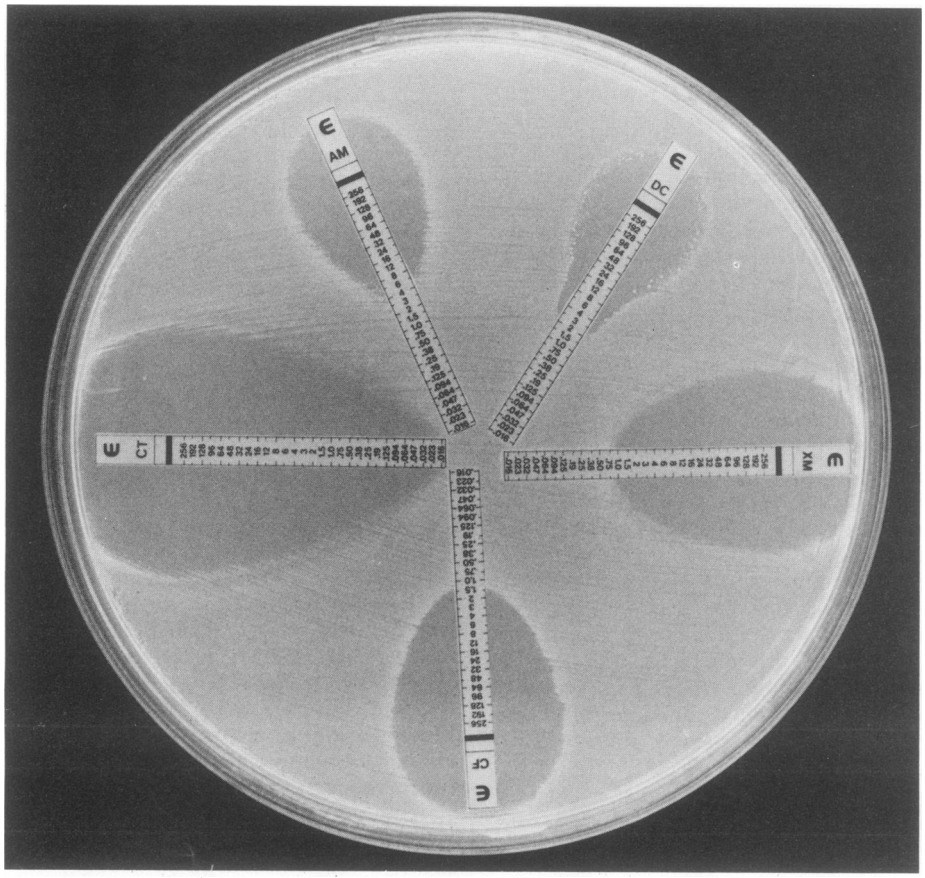

FIG. 1. E-tests with a β-lactamase-producing *H. influenzae* strain performed with HTM agar. Antibiotic abbreviations on the E-test strips and MIC interpretations (indexed to base 1) are AM (ampicillin; 8 μg/ml), DC (doxycycline; 4 μg/ml), XM (cefuroxime; 1 μg/ml), CF (cefaclor; 2 μg/ml), and CT (cefotaxime; 0.015 μg/ml).

pensions were prepared as described above and then diluted in Mueller-Hinton broth and delivered to the surface of the agar plates with a Steers replicator, which resulted in a final inoculum of ca. 10^4 CFU per spot. Agar dilution plates were incubated at 35°C in 5% CO_2 for 16 to 20 h before interpretation of the MICs in the usual manner (12).

RESULTS

One hundred *H. influenzae* and 50 *S. pneumoniae* strains were examined by E-tests performed on the agar media currently recommended for disk diffusion testing of those species by the NCCLS (HTM and Mueller-Hinton sheep blood agars, respectively), and those results were compared with the results of broth microdilution MIC tests performed with the media currently recommended by the NCCLS (HTM and Mueller-Hinton lysed horse blood broths, respectively). E-tests were also performed on a subset of 50 *Haemophilus* strains (including predominantly those strains with known resistance mechanisms) with the medium (PDM ASM II chocolate agar) recommended by the manufacurer of the E-test and by agar dilution with the same medium. E-test MICs were easily interpreted for most of the drugs with either of the agar media with *H. influenzae* and with Mueller-Hinton sheep blood agar with *S. pneumoniae*. The E-test inhibition ellipses were generally clearly demarcated, and the points of intersection of the zone edge with the strips were generally easily interpreted (Fig. 1). However, trimethoprim-sulfamethoxazole with both species and ampicillin with *H. influenzae* were exceptions. Some β-lactamase-producing *H. influenzae* yielded growth of numerous small colonies within the inhibition ellipse with ampicillin (Fig. 2). E-test MICs with such strains could be interpreted in several different ways (see Discussion). Inhibition ellipse margins with the trimethoprim-sulfamethoxazole combination were sometimes diffuse and poorly defined with *H. influenzae* on ASM II chocolate agar and with pneumococcal tests on Mueller-Hinton sheep blood agar. The problem was less pronounced with *H. influenzae* tested with HTM agar.

Despite the difficulty in interpreting the E-test MICs for the two drugs mentioned above, the overall agreement between E-test MICs and MICs determined by the conventional dilution methods was generally good. The overall agreement between E-test MICs for *H. influenzae* measured on HTM agar and microdilution MICs measured in HTM broth was 89.8% (Table 2). The highest agreement occurred with erythromycin tests (99.0%), and the lowest agreement values were seen with trimethoprim-sulfamethoxazole (67.7%) and ampicillin (76.5%) tests. The overall agreement between E-test MICs determined with ASM II chocolate agar and microdilution MICs determined with HTM broth was lower, i.e., 81.4% (Table 3), principally because of an even lower correlation between trimethoprim-sulfamethox-

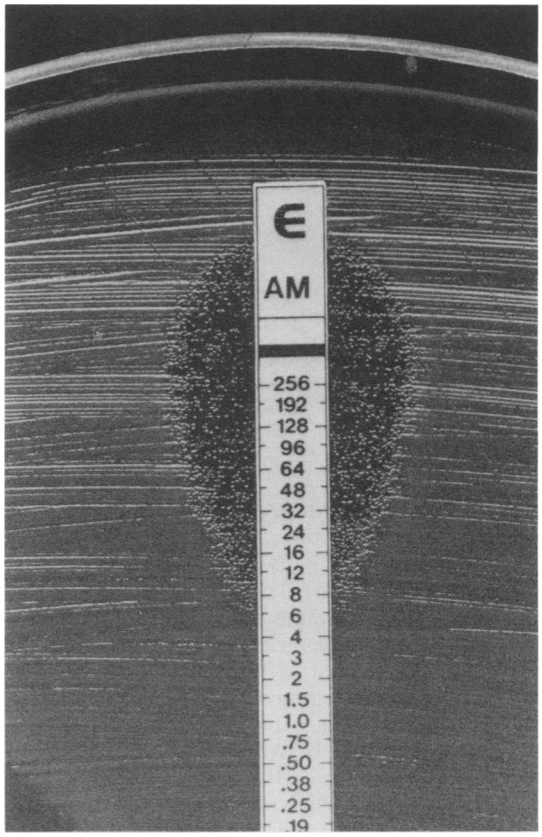

FIG. 2. Ampicillin E-test of a β-lactamase-producing *H. influenzae* strain performed with ASM II chocolate agar. The MIC was interpreted as >256 μg/ml because of the in-growth colonies.

TABLE 2. Comparison of *H. influenzae* E-test MICs determined on HTM agar with HTM broth microdilution MICs

Drug	No. of strains[a]	No. of E-test MICs within indicated concn (log_2) of HTM broth dilution MICs							% Agreement within 1 log_2 concn
		>−2	−2	−1	Same	+1	+2	>+2	
Ampicillin	98	7	13	14	37	24		3	76.5[b]
Cefaclor	100	4	8	31	43	13	1		87.0
Cefuroxime	100	2		6	53	36	3		95.0
Cefotaxime	100		4	24	59	13			96.0
Chloramphenicol	100		5	27	57	11			95.0
Doxycycline	100		3	11	44	40	2		95.0
Tetracycline	100		4	25	52	19			96.0
Erythromycin	100			22	53	24	1		99.0
Trimethoprim-sulfamethoxazole[c]	96		1	3	17	45	27	3	67.7
Overall agreement									89.8

[a] Indicates number of strains in which both tests resulted in a finite MIC; off-scale values were excluded from comparison.
[b] Twelve errors in 23 tests (>±1 log_2 dilution) with β-lactamase-positive isolates.
[c] Trimethoprim-sulfamethoxazole ratio, 1:19.

TABLE 3. Comparison of E-test MICs determined on ASM II agar with HTM broth microdilution MICs

Drug	No. of strains[a]	No. of E-test MICs within indicated concn (log_2) of HTM broth dilution MICs							% Agreement within 1 log_2 concn
		>−2	−2	−1	Same	+1	+2	>+2	
Ampicillin	50	7	5	19	13	5		1	74.0
Cefaclor	50	3	4	26	13	3	1		84.0
Cefuroxime	50			5	24	19	1	1	96.0
Chloramphenicol	50		1	20	21	8			98.0
Doxycycline	50			2	8	18	22		56.0
Tetracycline	50		1	4	20	20	5		88.0
Erythromycin	50			19	23	7	1		98.0
Trimethoprim-sulfamethoxazole[b]	47			2	5	19	19	2	55.3
Overall agreement									81.4

[a] Indicates number of strains in which both tests resulted in a finite MIC; off-scale values were excluded from comparison.
[b] Trimethoprim-sulfamethoxazole ratio, 1:19.

azole results (55.3%) and a poor correlation of doxycycline results (56.0%). With the exception of low agreement between trimethoprim-sulfamethoxazole results (67.4%), the most favorable correlation (91.8%) was seen between E-tests performed on ASM II chocolate agar and agar dilution MICs determined with the same medium (data not depicted). The agreement between E-test MICs with *H. influenzae* determined on the two different agars (HTM and ASM II chocolate) was 90.8% (Table 4).

The overall agreement between E-test and NCCLS reference broth microdilution MICs with *S. pneumoniae* was 80.4% (Table 5). The highest agreement occurred with chloramphenicol (98.0%), and the lowest agreement was seen with trimethoprim-sulfamethoxazole (42.0%). If the trimethoprim-sulfamethoxazole values were excluded, the overall agreement between E-test and broth microdilution MICs with pneumococci increased to 90%.

Interpretive category errors resulting from E-tests of both species were generally low (Table 6). Overall, only 0.7% of the E-tests had very major errors and none had major errors in the case of *H. influenzae* E-tests performed on HTM agar compared with the interpretive guidelines for the NCCLS reference broth microdilution test (12). Thus, the E-test MICs accurately categorized the *Haemophilus* strains which were resistant to the various study drugs. Likewise, the overall error rates of E-tests with pneumococci were relatively low, i.e., 0.8% of the tests had very major and 2.4% had major errors with a variety of interpretive criteria for the reference MICs (Table 6). No very major or major errors occurred with penicillin E-tests of pneumococci in comparison with the NCCLS criteria (11). Two very major errors (4%) occurred with tetracycline E-tests of pneumococci if an MIC of ≥16 μg/ml was regarded as indicative of tetracycline resistance.

A few other drugs yielded lower than average agreement between the E-test and conventional methods. Cefaclor and cefuroxime tests with *H. influenzae* resulted in 2% of the tests having very major errors and, in the case of cefaclor, 11% of the tests having minor errors (Table 6). E-tests of the quality control strains included in this study yielded MICs within the acceptable ranges for all of the study drugs.

DISCUSSION

The E-test represents a new and innovative approach to the determination of antimicrobial susceptibility which is potentially applicable to a wide array of drugs and microorganisms. In a manner reminiscent of the agar disk diffusion method, the E-test provides quantitative MICs simply and reproducibly. The E-test approach may be well suited to the testing of certain fastidious bacteria (e.g., *H. influenzae* and pneumococci) or bacteria that are difficult to test (e.g., anaerobes [1]). The present study has demonstrated the potential of the E-test for use with *H. influenzae* and *S. pneumoniae* with the media most often used to test these two species in the United States, i.e., HTM agar for *H. influ-*

TABLE 4. Comparison of *H. influenzae* E-test MICs determined on HTM agar with E-test MICs determined on ASM II chocolate agar

Drug	No. of strains[a]	No. of E-test MICs within indicated concn (log_2) of ASM II agar E-test MICs							% Agreement within 1 log_2 concn
		>−2	−2	−1	Same	+1	+2	>+2	
Ampicillin	50		2	6	19	22	1		94.0
Cefaclor	50	2		4	28	14	2		92.0
Cefuroxime	50	2		4	28	16			96.0
Chloramphenicol	50		1	15	32	2			98.0
Doxycycline	50	1	19	26	4				60.0
Tetracycline	50		5	43	2				90.0
Erythromycin	50			2	33	14	1		98.0
Trimethoprim-sulfamethoxazole[b]	47		1	5	32	8	1		95.7
Overall agreement									90.4

[a] Indicates number of strains in which both tests resulted in a finite MIC; off-scale values were excluded from comparison.
[b] Trimethoprim-sulfamethoxazole ratio, 1:19.

TABLE 5. Comparison of *S. pneumoniae* E-test MICs with broth microdilution MICs

Drug	No. of strains	No. of E-test MICs within indicated concn (log_2) of broth microdilution MICs							% Agreement within 1 log_2 concn
		>−2	−2	−1	Same	+1	+2	>+2	
Penicillin	50		13	31	6				74.0
Chloramphenicol	50			10	35	4	1		98.0
Tetracycline	50			14	26	7	3		94.0
Erythromycin	50		1	5	21	21	2		94.0
Trimethoprim-sulfamethoxazole[a]	50				2	19	21	8	42.0
Overall agreement									80.4

[a] Trimethoprim-sulfamethoxazole ratio, 1:19.

enzae and Mueller-Hinton sheep blood agar for *S. pneumoniae*. ASM II chocolate agar was also used for E-tests of *H. influenzae*, since it is the medium recommended by the manufacturer of the E-test strips and is sold for that purpose in Europe. However, the correlation between E-tests performed on that medium and HTM microdilution susceptibility tests was less favorable, and only slightly better overall agreement was noted between E-test and conventional agar dilution MICs determined by using the ASM II agar in contrast to the HTM agar recommended by the NCCLS.

E-test MICs were generally easily interpreted with both *Haemophilus* and pneumococcus isolates. Since E-test results are interpreted from the upper surface of the agar with the plate lid removed, there is no particular disadvantage in using opaque media such as blood- or hemoglobin-supplemented agars. The major exceptions were the diffuse growth ellipses encountered with the two blood- or hemoglobin-containing agars and trimethoprim-sulfamethoxazole E-tests. The indistinct endpoints with this antimicrobial combination resulted in a high rate of disagreement with the conventional dilution test results. The likely presence of folate metabolism antagonists in the hemoglobin supplement added to the ASM II agar and the sheep blood added to the Mueller-Hinton agar may account for the poorly delineated growth ellipses which made it difficult to interpret accurately the MIC endpoints. A further confounding factor was the preparation and labeling of the E-test strips for trimethoprim-sulfamethoxazole with the arithmetic total drug content, rather than only the trimethoprim component, indexed to the base 1, as is more common in the United States. Thus, when the 1:19 trimethoprim-to-sulfamethoxazole test ratio was considered, the trimethoprim-sulfamethoxazole MIC results did not coincide exactly. For example, the reference dilution MICs contained trimethoprim and sulfamethoxazole concentrations such as 4 and 76, 2 and 38, and 1 and 19 μg/ml, while the nearest E-test concentrations were 3.2 and 60.8 μg/ml (labeled 64 on the test strip), 1.6 and 30.4 μg/ml (labeled 32), and 0.8 and 15.2 μg/ml (labeled 16). Thus, any discordance between the E-test and reference methods was no doubt worsened by the inequitable concentration increments which were used for comparison. Despite the difficulties, there were no very major or major interpretive errors encountered with *H. influenzae*. However, the folate antagonists apparently present in the sheep blood resulted in substantial (12%) major errors in pneumococcal tests.

TABLE 6. Categorization of interpretive errors of E-test MICs[a] compared with broth microdilution MICs

Species and drugs	% Tests having errors of indicated degree		
	Very major	Major	Minor
H. influenzae[b]			
Ampicillin	1.0	0	1.0
Cefaclor	2.0	0	11.0
Cefuroxime	2.0	0	0
Cefotaxime	0	0	0
Chloramphenicol	0	0	0
Tetracycline	0	0	3
Trimethoprim-sulfamethoxazole[c]	0	0	2
Overall	0.7	0	2.4
S. pneumoniae			
Penicillin[b]	0	0	28.0
Chloramphenicol[d]	0	0	NA[e]
Tetracycline[f]	4.0	0	NA
Erythromycin[g]	0	0	NA
Trimethoprim-sulfamethoxazole[c,h]	0	12.0	NA
Overall	0.8	2.4	NA

[a] E-tests performed on HTM agar with *H. influenzae* and Mueller-Hinton sheep blood agar with *S. pneumoniae*.
[b] Based on interpretive criteria of NCCLS standard M7-A2 (12).
[c] Trimethoprim-sulfamethoxazole ratio, 1:19.
[d] Based on resistance at MIC of ≥8 μg/ml (10).
[e] NA, Not applicable.
[f] Based on resistance at MIC of ≥16 μg/ml.
[g] Based on resistance at MIC of ≥8 μg/ml.
[h] Based on resistance at MIC of ≥4 μg/ml.

The problem of in-growth colonies which occurred with ampicillin and β-lactamase-producing *H. influenzae* meant that E-test MICs could be read where the most obvious ellipse intersected the test strip (ignoring the in-growth colonies) or where the in-growth colonies diminished appreciably (the approach used in this study), or the MIC could be recorded as equal to or greater than the highest concentration on the test strip if some colonies occurred throughout most of the ellipse. The last option is recommended by the manufacturer of the E-test. That strategy reduces the possibility of false susceptibility (1% of tests had very major errors in this study) but does not improve agreement with the exact ampicillin MICs obtained by either conventional broth or agar dilution methods.

E-test MICs compared favorably with those of most of the other drugs tested by conventional methods against *H. influenzae* and with all but penicillin and trimethoprim-sulfamethoxazole against *S. pneumoniae*. It is not immediately clear why the penicillin MICs for pneumococci did not agree more closely. There was a trend toward somewhat higher (1 to 2 log_2 units) penicillin MICs determined by the

Mueller-Hinton lysed horse blood broth microdilution method compared with the E-test MICs determined on Mueller-Hinton sheep blood agar. Another study has shown recently that penicillin MICs for pneumococci were slightly higher in lysed horse blood broth than in a second microdilution test medium, HTM (5). The slightly lower penicillin E-test values did not result in any very major errors in this study; however, there were strains with reference penicillin MICs in the resistant range (i.e., ≥ 2 μg/ml) which were considered relatively resistant (MIC, 0.12 to 1 μg/ml) by E-tests, and likewise strains which were relatively resistant to penicillin by the reference method but susceptible (MIC, ≤ 0.06 μg/ml) by the E-test. While these differences are strictly classified as minor errors, they would lead to important differences in the categorization of pneumococcal penicillin resistance rates, and thus they seriously question the utility of the E-test for penicillin determinations with *S. pneumoniae*.

The E-test method represents a potential alternative for convenient performance of quantitative (MIC) susceptibility tests of *H. influenzae* and *S. pneumoniae*. With a few important exceptions noted above, E-test MICs correlated well with conventional MICs and with existing interpretive categories. The E-test method may be a convenient means for testing fastidious or anaerobic bacteria against a limited array of drugs. Since no more than six E-test strips can be placed on the surface of the 150-mm-diameter round petri plates most often used for susceptibility testing in the United States, the E-test will probably not represent an economical method for testing a large number of drugs on each isolate. The cost effectiveness of this new method has not been addressed in this article, since the final market price of the strips in the United States has not yet been decided by the manufacturer. Further studies are needed to fully explore the potential of this new method for antimicrobial susceptibility testing.

REFERENCES

1. **Citron, D. M., M. I. Ostovari, A. Karlsson, and E. J. C. Goldstein.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 876.
2. **Doern, G. V., and R. N. Jones.** 1988. Antimicrobial susceptibility testing of *Haemophilus influenzae*, *Branhamella catarrhalis*, and *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **32**:1747–1753.
3. **Doern, G. V., J. H. Jorgensen, C. Thornsberry, D. A. Preston, T. Tubert, J. S. Redding, and L. A. Maher.** 1988. National collaborative study of the prevalence of antimicrobial resistance among clinical isolates of *Haemophilus influenzae*. Antimicrob. Agents Chemother. **32**:180–185.
4. **Doern, G. V., J. H. Jorgensen, C. Thornsberry, and H. Snapper.** 1990. Disk diffusion susceptibility of *Haemophilus influenzae* using *Haemophilus* test medium—description of a method, zone diameter interpretive criteria and MIC correlates. Eur. J. Clin. Microbiol. Infect. Dis. **9**:329–336.
5. **Jorgensen, J. H., L. A. Maher, and A. W. Howell.** 1990. Use of *Haemophilus* test medium for broth microdilution antimicrobial susceptibility testing of *Streptococcus pneumoniae*. J. Clin. Microbiol. **28**:430–434.
6. **Jorgensen, J. H., J. S. Redding, L. A. Maher, and A. W. Howell.** 1987. Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. J. Clin. Microbiol. **25**:2105–2113.
7. **Klugman, K. P.** 1990. Pneumococcal resistance to antibiotics. Clin. Microbiol. Rev. **3**:171–196.
8. **Latorre, C., T. Juncosa, and I. Sanfeliu.** 1985. Antibiotic resistance and serotypes of 100 *Streptococcus pneumoniae* strains isolated in a children's hospital in Barcelona, Spain. Antimicrob. Agents Chemother. **28**:357–359.
9. **Machka, K., I. Braveny, H. Dabernat, K. Dornbusch, E. VanDyck, F. H. Kayser, B. Van Klingeren, H. Mittermayer, E. Perea, and M. Powell.** 1988. Distribution and resistance patterns of *Haemophilus influenzae*: a European cooperative study. Eur. J. Clin. Microbiol. Infect. Dis. **7**:14–24.
10. **Matthews, H. W., C. N. Baker, and C. Thornsberry.** 1988. Relationship between in vitro susceptibility test results for chloramphenicol and production of chloramphenicol acetyltransferase by *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Aerococcus* species. J. Clin. Microbiol. **26**:2387–2390.
11. **Mendelman, P. M., D. O. Chaffin, T. L. Stull, C. E. Rubens, K. D. Mack, and A. L. Smith.** 1984. Characterization of non-β-lactamase-mediated ampicillin resistance in *Haemophilus influenzae*. Antimicrob. Agents Chemother. **26**:235–244.
12. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
13. **National Committee for Clinical Laboratory Standards.** 1990. Performance standards for antimicrobial disk susceptibility tests. Tentative standard M2-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.