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Evaluation of Commercial Methods for Determining Antimicrobial Susceptibility of *Streptococcus pneumoniae*

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Seven commercial systems for antimicrobial susceptibility testing of *Streptococcus pneumoniae* were evaluated by using a challenge set of 55 pneumococcal isolates with a variety of resistance phenotypes and genotypes. Overall, the results produced by the Pasco and Etest methods were found to be acceptable for all drugs tested except for trimethoprim-sulfamethoxazole testing by the Etest. The Just One system for penicillin MIC testing was also judged to be acceptable (minor error rate, 5.5%). Although the Sensititre and MicroTech methods both produced 12.7% minor errors with penicillin, the Sensititre method classified penicillin-intermediate strains as resistant or vice versa, while four of MicroTech's errors were among intermediate strains that were classified as susceptible. The MicroMedia (minor error rate, 16.4%) and MicroScan Rapid (minor error rate, 63.6%) methods produced unacceptably high levels of errors when testing penicillin. Minor error rates for cefotaxime and ceftriaxone ranged from a low of 12.7% (Etest and Sensititre) to a high of 28% (MicroMedia). Error rates were low for erythromycin, tetracycline, and chloramphenicol by most methods with the exception of the MicroScan method, which had a high very major error rate for erythromycin (34.6%). For testing of β-lactam drugs, the Pasco, Etest, and Just One tests for penicillin are the most accurate methods; the Sensititre method also provided acceptable results.

Strains of *Streptococcus pneumoniae*, particularly those resistant to antimicrobial agents, continue to be a major cause of otitis media, pneumonia, and meningitis in the United States (2, 8, 10, 29, 32) and around the world (7, 19, 22, 33). Therapeutic regimens for pneumococcal disease have been compromised over the last decade by the development of resistance to penicillin (9, 12, 16, 17), extended-spectrum cephalosporins (4, 13, 31), and other antimicrobial agents (19, 23, 24, 33). A recent report from Atlanta emphasized that 25% of isolates from patients with invasive pneumococcal disease were no longer susceptible to penicillin and 9% were no longer susceptible to cefotaxime (10). Thus, determining the susceptibilities of pneumococcal isolates to therapeutic drugs is important, particularly in cases of invasive disease (8, 32).

While several studies have evaluated the abilities of commercial methods, such as the Etest (11, 14, 21, 27), Just One for penicillin (18), the MicroScan type 6 panel (5, 18, 28, 30), and the MicroMedia FOX panel (3, 5, 20), to detect antimicrobial resistance in pneumococci, those studies have often been limited to one or two methods, and they frequently have not included the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution reference method (25) for comparison. In addition, several of the studies only evaluated the accuracy of the penicillin MIC results.

Because of the increasing emphasis on the importance of determining local rates of pneumococcal resistance to guide empiric therapy for invasive disease (2, 4), we undertook the study described here to assess the accuracies of commercially available methods of pneumococcal susceptibility testing. We compared the MIC data obtained with seven commercial systems with the MIC data generated by the NCCLS broth microdilution reference method for a challenge set of 55 pneumococcal strains. The results of the study are presented here.

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MATERIALS AND METHODS

Bacterial strains. Fifty-five isolates of *S. pneumoniae* from the culture collection of the Centers for Disease Control and Prevention were selected for testing. The organisms were chosen so that at least 20 isolates were resistant to each of the drugs evaluated. The organisms were identified by standard reference procedures (6). *S. pneumoniae* ATCC 49619 was used for quality control of all susceptibility testing methods.

Systems evaluated. The following methods were evaluated in the present study: Etest (AB Biodisk, Piscataway, N.J.), Just One (for penicillin only; AcuMed, Westlake, Ohio), MicroScan rapid panels (Dade International, West Sacramento, Calif.), MicroMedia (AcuMed), MicroTech (Aurora, Colo.), Pasco (Difco, Wheatridge, Colo.), and Sensititre (AcuMed). Lysed horse blood was obtained from Carr-Scarborough Microbiologicals, Inc. (Decatur, Ga.), for use with the Sensititre test. The blood required centrifugation as described by NCCLS (25) before it was used. The MicroScan and MicroMedia panels both use proprietary broth formulations that do not require supplementation with lysed horse blood, while the MicroTech panels are supplemented with lysed horse blood by the manufacturer. For the Pasco panels, the inoculum was prepared in a lysed horse blood supplement provided by the manufacturer. The Etest was performed on Mueller-Hinton agar containing 5% sheep blood (Becton Dickinson Microbiology Systems, Cockeysville, Md.). The panels and disposables for the study were donated by each of the manufacturers. Any Etest MIC results that were between doubling dilutions were rounded up to the next doubling dilution MIC for data analysis. All test plates were incubated in ambient air at 35°C except for the plates containing the Etest strips, which were incubated at 35°C in 5% CO_2 as recommended by the manufacturer.

Reference method. The reference method for the present study was the NCCLS broth microdilution test with cation-adjusted Mueller-Hinton broth (Difco, Detroit, Mich.) containing 5% lysed horse blood (25). Lysed horse blood was prepared as described by NCCLS (25). The interpretive criteria and quality control ranges were those described by Jorgensen et al. (15) and published in NCCLS document M100-S5 (26). Microdilution plates were incubated in ambient air at 35°C and were read after 20 to 24 h. For all strains for which the tests showed very major or major errors, the strains were repeat tested once by the reference method and in duplicate by the test method in question. MICs for *S. pneumoniae* ATCC 49619 were determined on each testing day. No quality control results were out of range during the course of the study.

TABLE 1. Baseline data on the distribution of antimicrobial susceptibility patterns of the 55 pneumococcal study isolates tested by the reference broth microdilution method

Antimicrobial agent	No. of strains with the following interpretive categories[a]:		
	S	I	R
Penicillin	11	14	30
Cefotaxime	24	6	25
Ceftriaxone[b]	24	9	22
Chloramphenicol	33		22
Erythromycin[b]	28	1	26
Tetracycline	30	0	25
Trimethoprim-sulfamethoxazole	13	6	36

[a] S, susceptible; I, intermediate; R, resistant.
[b] The baseline reference MIC data used to evaluate the Sensititre panels were generated on a testing day separate from the day on which the data provided here were obtained and showed slightly different results (see text). Two strains that were initially classified as ceftriaxone intermediate by the reference method were reclassified as susceptible when they were retested (in each case there was only a 1 doubling dilution difference between the results), and one strain initially classified as erythromycin intermediate was reclassified as resistant when it was retested (also a 1 dilution difference).

RESULTS

Selection of isolates and stability of the broth microdilution reference method. The pneumococcal isolates used in the study were chosen from the strain collection of the Centers for Disease Control and Prevention to represent a wide range of resistance phenotypes and genotypes to challenge the ability of the commercial systems to detect resistance. The strains overrepresented the percentage of resistant pneumococci that would normally be observed in most clinical laboratories. The interpretive categories (susceptible, intermediate, and resistant) determined by the reference method for the study strains are provided in Table 1. Since Sensititre panels were unavailable on the first day of testing, a second set of reference results were generated for use with the Sensititre method only. The reference MICs for several organisms changed by a single dilution on retesting, which changed their interpretive category. However, the end result was only apparent for the reference values for Sensititre panels for ceftriaxone (two strains) and erythromycin (one strain), which are slightly different from those for the other methods (Table 1). The category interpretations for all other drugs were the same for the 2 initial testing days. Each of the pneumococcal strains was tested at least three times by the reference method with the seven antimicrobial agents.

Overall, the reproducibility of the reference method was very good, with only 0.48% of MICs falling outside the range of ±1 dilution of the modal MIC. For penicillin, ceftriaxone, and trimethoprim-sulfamethoxazole, all reference test MIC results were within 1 dilution of the modal MIC. On a single occasion for one strain each tested with cefotaxime, tetracycline, and chloramphenicol, the MIC was 2 dilutions lower than the modal MIC. Although for four pneumococcal isolates erythromycin MICs by the reference test on one testing day were either 2 or, in one case, 3 dilutions lower than the modal MIC, none of these produced categorical errors. Thus, the categorical interpretations and the actual MICs determined by the broth microdilution MIC tests were highly reproducible.

Penicillin testing. The results of penicillin testing are given in Table 2. The Pasco system showed only one minor error (1.8%) for penicillin: the MIC for a strain for which the reference MIC was 2 μg/ml (resistant) was reported to be 1 μg/ml (intermediate). This error is designated R→I, where R represents a result indicating resistance by the reference method and I represents a result indicating intermediate susceptibility by the test method. The Etest showed two minor errors (3.6%). In both cases, the Etest result was 1 dilution higher than the result by the reference method. There was one S→I error (where S represents a result indicating susceptibility) and one I→R error. The Just One strip showed three minor errors (5.5%): one S→I error, one R→I error, and one I→R error. The highest MIC on the Just One strip is 1 μg/ml, so for resistant strains MICs are recorded as >1 μg/ml.

The Sensititre method showed seven minor errors (12.7%): four S→I errors and three I→R errors. In each case the results were 1 dilution higher than those obtained by the reference method. Seven minor errors were also observed by the MicroTech method, although they were not with the same seven organisms that were misclassified by the Sensititre method. The results obtained by the MicroTech method were reproducibly 1 dilution lower than those obtained by the reference method, showing four I→S errors and three R→I errors. For both systems the errors were made with strains for which MICs were consistent by the reference method. The MicroMedia method showed nine minor errors (16.4%); six S→I errors, two R→I errors, and one I→R error. Finally, the MicroScan method demonstrated 35 (63.6%) minor errors. All of the erroneous results were lower than the reference results. Seven results were 3 dilutions lower, 22 were 2 dilutions lower, and the remainder were a single dilution lower.

Cefotaxime testing. The results of cefotaxime testing are given in Table 3. There were more errors with this drug than with penicillin. Pasco showed 11 minor errors (20%); in every case these errors were 1 dilution lower than the reference MIC. In two cases, the errors were I→S; for nine strains, resistant strains were reported as intermediate. The Etest showed eight minor errors (14.5%); these errors were each 1 dilution lower than the MICs obtained by the reference method. Two were I→S errors and the remainder were R→I errors. The MicroTech method showed 10 minor errors with cefotaxime (18.2%). They were consistently 1 dilution lower than the MICs obtained by the reference method. All were R→I errors. MicroMedia demonstrated 7 (28%) very major errors with cefotaxime and 11 (20%) minor errors. The Mi-

TABLE 2. Error rates observed by various methods when testing pneumococci for penicillin resistance

Method	Range (μg/ml)[a]	No. (%) of errors			On scale (%)[b]	Off scale[c]	% ±1 dilution (n)[d]
		Very major	Major	Minor			
Pasco	0.03–2	0	0	1 (1.8)	63.6	4/16	97.4 (39)
Etest	0.002–32	0	0	2 (3.6)	94.5	0/3	94.2 (52)
Just One	0.015–1	0	0	3 (5.5)	41.8	1/30	91.3 (23)
Sensititre	0.03–8	0	0	7 (12.7)	92.7	0/4	96.8 (50)
MicroTech	0.015–2	0	0	7 (12.7)	74.5	4/10	100 (41)
MicroMedia	0.015–2	0	0	9 (16.4)	70.9	1/15	97.4 (39)
MicroScan	0.03–8	0	0	35 (63.6)	67.3	15/3	29.7 (37)

[a] Dilution range of penicillin on the test panel.
[b] On scale indicates percentage of MICs within the dilution range on the test panel.
[c] Off scale results indicate the number of isolates for which MICs are below the lowest dilution on the test panel/number of isolates for which MICs are above the highest dilution on the test panel.
[d] Percentage of on-scale MICs within ±1 dilution of the broth microdilution reference method; numbers in parentheses are the number of on-scale values used in the calculation.

TABLE 3. Error rates observed by various methods when testing pneumococci for cefotaxime resistance

Method	Range (μg/ml)[a]	No. (%) of errors			On scale (%)[b]	Off scale[c]	% ±1 dilution (n)[d]
		Very major	Major	Minor			
Pasco	0.03–2	0	0	11 (20)	61.8	9/12	100 (34)
Etest	0.002–32	0	0	8 (14.5)	100	0/0	100 (53)
MicroTech	0.25–2	0	0	10 (18.2)	40.0	21/12	100 (22)
MicroMedia	0.5–32	7 (28.0)	0	11 (20.0)	34.5	19/0	89.5 (19)

[a] Dilution range of cefotaxime on the test panel.
[b] See footnote *b* of Table 2.
[c] See footnote *c* of Table 2.
[d] See footnote *d* of Table 2.

TABLE 5. Error rates observed by various methods when testing pneumococci for chloramphenicol resistance

Method	Range (μg/ml)[a]	No. (%) of errors			On scale (%)[b]	Off scale[c]	% ±1 dilution (n)[d]
		Very major	Major	Minor			
Pasco	1–16	0	0	0	80.0	0/11	100 (44)
Sensititre	0.5–32	0	1 (3.0)	0	100	0/0	98.2 (55)
MicroTech	2–16	0	1 (3.0)	0	54.5	5/20	100 (30)
MicroMedia	0.25–16	0	0	0	100	0/0	100 (55)
MicroScan	4–16	2 (9.1)	0	0	29.1	35/4	93.8 (16)

[a] Dilution range of chloramphenicol on the test panel.
[b] See footnote *b* of Table 2.
[c] See footnote *c* of Table 2.
[d] See footnote *d* of Table 2.

croScan results could not be evaluated because the lowest dilution tested on the panel was 4 μg/ml.

Ceftriaxone testing. The results of ceftriaxone testing are given in Table 4. For Pasco, eight minor errors (14.5%) were observed. As with cefotaxime, each error was 1 dilution below the reference MIC. Only one error represented an intermediate strain that was susceptible by the reference method. There were seven minor errors (12.7%) with the Etest. Again, all but one of the errors were 1 dilution step lower than the reference MIC. Two were I→S errors, four were R→I errors, and one was an I→R error.

The Sensititre system showed seven minor errors (12.7%; five I→R errors and two S→I errors), but with one exception, the results matched those of the reference method on other testing days. The MicroTech system showed one very major error (4.5%) and eight minor errors (14.5%) with ceftriaxone. The minor errors, consistent with the results obtained by cefotaxime testing, were 1 dilution lower with one exception, in which a susceptible strain was reported as intermediate. The MicroScan results could not be evaluated because the lowest dilution tested on the panel was 4 μg/ml.

Chloramphenicol testing. The results of chloramphenicol testing are given in Table 5. No errors were noted with chloramphenicol and the Pasco and MicroMedia methods. One major error was observed with the Sensititre method (3%); this result was 1 dilution lower than the reference MIC.

The MicroTech system showed one major error (3%) with chloramphenicol, but this error resolved on repeat testing. The MicroScan system showed two very major errors (9.1%); both results were 2 dilutions lower than the reference results.

Erythromycin testing. The results of erythromycin testing are given in Table 6. The Pasco method showed only a single minor error (1.8%), in which the MIC by the Pasco method was 1 dilution higher than the MIC by the reference method. The Etest showed two minor errors (3.6%): one I→R error and one R→I error. A single minor error (1.8%) was observed with the Sensititre system (R→I). The MicroMedia system showed one very major error (3.9%) and three minor errors (5.5%), while the MicroScan system showed nine very major errors (34.6%) and three minor errors (5.5%). On repeat testing of the MicroScan system, only one of the very major errors resolved. The minor errors were 2 to 5 dilutions below the results by the reference method.

Tetracycline testing. The results of tetracycline testing are given in Table 7. There were no errors with the Pasco, Sensititre, MicroScan, or MicroTech systems, although with the last system none of the values (0 of 55) were on scale. The MicroMedia system showed a single very major error. Strains were not tested for tetracycline resistance by the Etest.

Trimethoprim-sulfamethoxazole testing. The results of trimethoprim-sulfamethoxazole testing are given in Table 8. There were three minor errors with the Pasco system (5.5%), in which intermediate strains were called resistant or vice versa. The Etest showed 21 minor errors (38.2%); 16 were 1 dilution lower and 5 were 2 dilutions lower than the MIC obtained by the reference method. There were 3 I→S errors and 18 R→I errors. With the Sensititre system 14 minor errors were observed: 13 were 1 dilution lower (R→I) and 1 was 2 dilutions higher (I→R) than the MICs obtained by the reference method. The MicroTech system showed three minor errors (5.5%): all were 1 dilution higher than the reference values (I→R errors). The MicroMedia and MicroScan systems do not test trimethoprim-sulfamethoxazole on their panels.

TABLE 4. Error rates observed by various methods when testing pneumococci for ceftriaxone resistance

Method	Range (μg/ml)[a]	No. (%) of errors			On scale (%)[b]	Off scale[c]	% ±1 dilution (n)[d]
		Very major	Major	Minor			
Pasco	0.015–2	0	0	8 (14.5)	78.2	2/10	100 (43)
Etest	0.002–32	0	0	7 (12.7)	100	0/0	98.1 (53)
Sensititre	0.015–2	0	0	7 (12.7)	70.9	2/14	100 (38)
MicroTech	0.25–2	1 (4.5)	0	8 (14.5)	45.5	21/9	96.0 (25)
MicroMedia	0.5–32	1 (4.5)	0	12 (21.8)	50.9	27/0	92.9 (28)

[a] Dilution range of ceftriaxone on the test panel.
[b] See footnote *b* of Table 2.
[c] See footnote *c* of Table 2.
[d] See footnote *d* of Table 2.

TABLE 6. Error rates observed by various methods when testing pneumococci for erythromycin resistance

Method	Range (μg/ml)[a]	No. (%) of errors			On scale (%)[b]	Off scale[c]	% ±1 dilution (n)[d]
		Very major	Major	Minor			
Pasco	0.015–4	0	0	1 (1.8)	58.2	0/23	100 (32)
Etest	0.016–256	0	0	2 (3.6)	74.5	0/14	22.5 (40)
Sensititre	0.25–16	0	0	1 (1.8)	25.5	28/13	100 (12)
MicroTech	0.5–4	0	0	0	7.3	28/23	100 (4)
MicroMedia	0.12–8	1 (3.9)	0	3 (5.5)	25.5	28/13	91.7 (12)
MicroScan	0.25–4	9 (34.6)	0	3 (5.5)	23.6	38/4	88.9 (9)

[a] Dilution range of erthromycin on the test panel.
[b] See footnote *b* of Table 2.
[c] See footnote *c* of Table 2.
[d] See footnote *d* of Table 2.

TABLE 7. Error rates observed by various methods when testing pneumococci for tetracycline resistance

Method	Range (μg/ml)[a]	No. (%) of errors			On scale (%)[b]	Off scale[c]	% ±1 dilution (n)[d]
		Very major	Major	Minor			
Pasco	0.06–8	0	0	0	50.9	2/25	100 (28)
Sensititre	0.25–32	0	0	0	45.5	28/2	100 (25)
MicroTech	1–8	0	0	0	0	30/25	0 (0)
MicroMedia	0.25–16	1 (4.0)	0	0	14.5	25/22	100 (8)
MicroScan	0.5–8,128	0	0	0	45.5	30/0	96.0 (25)

[a] Dilution range of tetracycline on the test panel.
[b] See footnote *b* of Table 2.
[c] See footnote *c* of Table 2.
[d] See footnote *d* of Table 2.

DISCUSSION

In pneumococci the rates of resistance to a variety of antimicrobial agents are increasing around the world (2, 4, 9, 10, 12, 16, 17, 33). However, until recently, relatively few microbiologists in the United States tested pneumococci for resistance, or they limited testing to the oxacillin screen test. This was done, in part, because many microbiologists believed that pneumococci in the United States were uniformly susceptible to antimicrobial agents and partially because of the perceived difficulty of using the NCCLS broth microdilution method, which requires the preparation of lysed horse blood. Now that resistance is more widespread (2, 4), it is important to determine rates of resistance locally so that empiric therapy can be altered appropriately. Several commercial methods of determining the susceptibility profiles of pneumococci are available; however, reports of their accuracy vary (3, 5, 11, 14, 18, 20, 21, 27, 28, 30). To date, no studies have compared all of the commercially available methods simultaneously against the NCCLS reference broth microdilution method. Therefore, we undertook the present study to help guide microbiologists in their choice of a susceptibility testing system for pneumococci.

In the present study, the Pasco system reported penicillin MICs that tended to be equal to or 1 dilution lower than those obtained by the NCCLS reference method. A report by Nolte et al. (28) noted that Pasco MIC panels did not support the growth of 86% of strains when a commercial lysed horse blood supplement was used (28). This problem was not observed in the present study since the manufacturer provided the supplement in which the inoculum was prepared. The Etest and Sensititre systems, on the other hand, tended to report penicillin MICs that were equal to or 1 dilution higher than those obtained by the reference method, which made some strains appear marginally more resistant to penicillin. While no penicillin-intermediate or -resistant strains were missed by the Sensititre system, the MicroMedia system, with nine minor errors, reported many more strains as resistant. A similar result was noted with the MicroMedia system in a study by Clark et al. (5). This can be a problem because a result of false resistance may lead to the use of vancomycin or other alternate, and perhaps less effective, drugs for treating serious pneumococcal infections. The MicroTech system, on the other hand, misclassified four penicillin-intermediate strains as susceptible, which is a more serious problem. MicroScan had a remarkable minor error rate of 63.6%, producing results that were frequently 2 to 3 dilutions lower than the reference results. This is the first evaluation of the use of MicroScan rapid panels for testing pneumococci for penicillin resistance. Previous studies by Clark et al. (5), Kiska et al. (18), Nolte et al. (28), and Shanholtzer and Peterson (30) all used MicroScan type 6 conventional panels, although similar errors, i.e., the inability to detect penicillin resistance, were noted in each of those studies. Because the MicroScan system had already withdrawn the conventional panels at the time that the present study was undertaken (1), the results obtained with the conventional panels are not reported here.

Testing of cefotaxime and ceftriaxone is becoming increasingly important as the rates of resistance to these drugs increase in pneumococci (4, 13, 31). Since the intermediate category is a single dilution, and for penicillin-resistant strains the MICs of the extended-spectrum cephalosporins cluster around the intermediate value, there are bound to be high error rates. Even the reference method showed occasional 1-dilution differences on retesting. However, it is important that resistant strains not go undetected, since cefotaxime and ceftriaxone are the major therapeutic modalities for pneumococcal meningitis and sepsis (8, 32). In this regard, the Pasco and Etest systems each called two cefotaxime-intermediate strains susceptible, but they had 100% on-scale values within ±1 dilution of the reference value. Macias et al. (21) reported similar results in their study of the Etest. The errors observed with the MicroTech method for cefotaxime were all with resistant strains that were reported as intermediate. These minor errors have less of an impact than those in which resistance is missed altogether, yet the high error rate is still of a concern. On the other hand, the MicroMedia system's results for cefotaxime were unacceptable since they showed a 28% very major error rate. Unfortunately, the MicroScan system's results for cefotaxime and ceftriaxone could not be evaluated because the lowest dilution on the panel (4 μg/ml) was above the NCCLS resistance breakpoint (2 μg/ml).

For ceftriaxone, the Pasco and Etest systems again produced acceptable results in that only one and two strains, respectively, were intermediate strains that were called susceptible by the test system. The Sensititre system's results also were acceptable for ceftriaxone, showing no false-susceptible results among its seven minor errors. (Sensititre panels do not contain cefotaxime.) The MicroMedia system's results showed both very major and minor errors that tended to call intermediate strains susceptible, as did the MicroTech method.

Of the non-β-lactam drugs tested in the laboratory, erythromycin may be the most important since NCCLS recommends that it be used to predict the activities of clarithromycin and azithromycin, which are often prescribed for both children and adults suspected of having noninvasive pneumococcal disease (8). The Pasco and Etest systems both performed well, showing low minor error rates. Previous studies by Jacobs et al. (11) with the Etest system also found it acceptable for erythromycin when testing pneumococci. The Sensititre system also

TABLE 8. Error rates observed by various methods when testing pneumococci for trimethoprim-sulfamethoxazole resistance

Method	Range (μg/ml)[a]	No. (%) of errors			On scale (%)[b]	Off scale[c]	% ±1 dilution (n)[d]
		Very major	Major	Minor			
Pasco	0.06/1.2–4/76	0	0	3 (5.5)	74.3	0/14	100 (41)
Etest	0.002–32	0	0	21 (38.2)	92.7	0/4	88.0 (50)
Sensititre	0.06/1.2–4/76	0	0	14 (25.5)	74.5	0/14	100 (41)
MicroTech	0.5/9.5–2/38	0	0	3 (5.5)	5.5	13/39	100 (3)

[a] Dilution range of trimethoprim-sulfamethoxazole on the test panel.
[b] See footnote *b* of Table 2.
[c] See footnote *c* of Table 2.
[d] See footnote *d* of Table 2.

showed low error rates, but with only 25.5% of the values being on scale, the accuracy of the test is difficult to determine. Similarly, the MicroTech system, while showing no errors, produced only four on-scale results.

All systems performed well with tetracycline and chloramphenicol and reasonably well with trimethoprim-sulfamethoxazole. The high minor error rate for trimethoprim-sulfamethoxazole with the Etest system was unexpected and may be related to different sources of sheep blood, although we do not have data to support this hypothesis.

In summary, the Pasco and Etest methods were highly accurate in the detection of resistance to β-lactam drugs and can be used with confidence. The Just One system also provided accurate results for penicillin. The Sensititre system had a relatively high rate of minor errors, but it did not misclassify any intermediate or resistant strains as susceptible. Thus, this system may be viewed as an acceptable alternative method. However, users must prepare their own lysed horse blood for addition to the panels.

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