Comparison of E Test with Standard Broth Microdilution for Determining Antibiotic Susceptibilities of Penicillin-Resistant Strains of *Streptococcus pneumoniae*

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We compared the E test (AB Biodisk North America, Inc., Culver City, Calif.) with the National Committee for Clinical Laboratory Standards broth microdilution method for the determination of MICs of penicillin and cefotaxime for 108 isolates of *Streptococcus pneumoniae*. The E test was performed following manufacturer’s recommendations with Mueller-Hinton blood agar, and the broth microdilution procedure was performed with lysed horse blood-supplemented Mueller-Hinton broth. The microdilution method classified 26 isolates as highly penicillin resistant (MIC, ≥2 μg/ml), 33 as intermediate resistant to penicillin (MIC, ≥0.1 and <2.0 μg/ml), and 49 as susceptible to penicillin (MIC, <0.1 μg/ml). Discordant results obtained with the E test for penicillin susceptibility testing compared with broth microdilution occurred for 19 of the 108 isolates tested. Cefotaxime MICs for 90% of isolates found highly resistant, intermediately resistant, and susceptible to penicillin by broth microdilution were 2.0, 0.5, and 0.06 μg/ml, respectively. There were 16 susceptibility category changes when the E test was used to determine cefotaxime MICs. All of the discrepancies in the penicillin and cefotaxime MICs determined by the E test occurred at the susceptibility category breakpoints, and all represented differences of only one twofold dilution factor. Properly performed and controlled, the E test should be a reliable quantitative procedure for more accurately predicting the susceptibility of *S. pneumoniae* to several antibiotics.

*MATERIALS AND METHODS*

**Bacterial isolates.** A total of 108 clinical isolates of *S. pneumoniae* were tested. Eighty-two isolates were obtained from Texas Children's Hospital, Houston. Twenty-six additional isolates were obtained from the Veteran's Administration Medical Center, Houston, Tex. (kindly provided by D. M. Musher), and included 3 American Type Culture Collection isolates. All isolates were identified as *S. pneumoniae* on the basis of susceptibility to optochin, solubility in bile salts, and positive reaction with serogroup-specific antisera.

**Antimicrobial susceptibility testing.** MICs for the isolates of *S. pneumoniae* were determined by the National Committee for Clinical Laboratory Standards (NCCLS)-recommended broth microdilution method (14) with in-house-prepared Mueller-Hinton broth supplemented with divalent cations and lysed horse blood to a final concentration of 3%. Isolates were designated susceptible to penicillin (Bristol Myers Squibb, Evansville, Ind.) when the MIC was <0.1 μg/ml, intermediately resistant when the MIC was between 0.1 and 2 μg/ml, and highly resistant when the MIC was ≥2 μg/ml. Cefotaxime standard powder was obtained from Hoechst-Roussel Pharmaceuticals Inc. (Somerville, N.J.). Guidelines proposed by the NCCLS for the interpretation of cefotaxime susceptibility, with the proviso that intermediate isolates cultured from central nervous system infections be reported as resistant (9), are as follows: susceptible, MIC of ≤0.25 μg/ml; intermediate, MIC of 0.5 to 1.0 μg/ml; and resistant, MIC of ≥2.0 μg/ml.

The E test was performed following the manufacturer's recommendations with Mueller-Hinton–sheep blood agar.
TABLE 1. Results of penicillin susceptibility testing by the E test and broth microdilution

<table>
<thead>
<tr>
<th>Broth microdilution result (no. of isolates tested)</th>
<th>No. of isolates categorized as follows by the E test:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>Resistant (26)</td>
<td>23</td>
</tr>
<tr>
<td>Intermediately resistant (33)</td>
<td>5</td>
</tr>
<tr>
<td>Susceptible (49)</td>
<td>0</td>
</tr>
</tbody>
</table>

* See the text for MIC definitions.

TABLE 2. Results of cefotaxime susceptibility testing by the E test and broth microdilution

<table>
<thead>
<tr>
<th>Broth microdilution result (no. of isolates tested)</th>
<th>No. of isolates categorized as follows by the E test:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>Resistant (8)</td>
<td>8</td>
</tr>
<tr>
<td>Intermediate (23)</td>
<td>4</td>
</tr>
<tr>
<td>Susceptible (77)</td>
<td>0</td>
</tr>
</tbody>
</table>

* See the text for MIC definitions.

plates (BBL Microbiology Systems, Cockeysville, Md.). In brief, *S. pneumoniae* was grown overnight on blood agar plates, suspended in Trypticase soy broth to a 0.5 MacFarland density, and inoculated for confluent growth onto Mueller-Hinton–blood agar plates. Penicillin and cefotaxime E test strips were applied to the plates after the inoculum had dried for 15 min. The penicillin and cefotaxime E test strips used had a MIC range of 0.002 to 32 μg/ml. Incubation was at 35°C, under 5% CO₂ for 18 to 24 h. The MICs were read at the point of intersection between the edge of the zone of bacterial growth and the E test strip, per manufacturer's instructions. The E test produces MICs by using a continuous scale that generates values that fall between conventional twofold dilutions. E test results were rounded up to the next higher twofold dilution value as recommended in the manufacturer's package insert.

Inoculum densities for the E test and broth microdilution assays were carefully controlled, and colony counting was performed for verification. The final cell concentration in each inoculum was 5 × 10⁵ CFU/ml.

RESULTS

The MICs of penicillin and cefotaxime were determined for 108 isolates of *S. pneumoniae* by broth microdilution and the E test. The microdilution method classified 26 isolates as highly penicillin resistant, 33 as intermediately resistant to penicillin, and 49 as susceptible to penicillin. Susceptibility category changes were observed for 19 of these isolates when the E test was used to determine the MICs (Table 1). The E test interpreted three isolates categorized as highly resistant to penicillin by broth microdilution as intermediately resistant. All of the discrepancies represented differences of one dilution and occurred at the breakpoint between 2.0 and 1.0 μg/ml. There were five discrepancies for isolates categorized as intermediately resistant by broth microdilution. All five isolates were classified as highly resistant by the E test, all at the breakpoint between 2.0 and 1.0 μg/ml. None of the isolates classified as intermediately resistant by broth microdilution were classified as susceptible by the E test. Eleven of the 49 isolates categorized as susceptible to penicillin by broth microdilution were interpreted as having intermediate resistance to penicillin by the E test. All 11 discrepancies occurred at the breakpoint between 0.06 and 0.125 μg/ml.

Cefotaxime MICs for 90% of isolates found susceptible, intermediately resistant, and highly resistant to penicillin by broth microdilution were 0.06, 0.5, and 2.0 μg/ml, respectively; the corresponding values with the E test were 0.125, 0.5, and 3.0 μg/ml. There were 16 susceptibility category changes (Table 2) when cefotaxime was evaluated by the E test and the results were compared with the results obtained by broth microdilution. Eight isolates were determined to be resistant to cefotaxime by both methods. All but one of these eight cefotaxime-resistant isolates were highly resistant to penicillin. Twenty-three isolates were found intermediately resistant to cefotaxime by broth microdilution. Four of these isolates were categorized as resistant to cefotaxime by the E test, and the E test MIC differed from the broth microdilution MIC by two dilution factors. One isolate was classified as intermediately resistant to cefotaxime by broth microdilution but susceptible by the E test. Eleven isolates determined to be susceptible by broth microdilution were categorized as intermediately resistant by the E test. All of the discrepancies represented differences of one dilution and occurred at the breakpoint between 0.5 and 0.25 μg/ml.

DISCUSSION

Accurate and rapid assessment of the antimicrobial susceptibility of *S. pneumoniae* has become a major concern because of the increasing incidence of resistant isolates reported worldwide. Routine penicillin susceptibility screening with a 1-μg oxacillin disk on sheep blood-supplemented Mueller-Hinton agar fails to distinguish between *S. pneumoniae* isolates highly resistant to penicillin (MIC, ≥2.0 μg/ml) and isolates intermediately resistant to penicillin (MIC, ≥0.1 and <2.0 μg/ml) (10, 17). In addition, the increasing incidence of resistance to other antimicrobial agents in addition to penicillin mandates expanded susceptibility testing. The E test, a paper strip impregnated with a linear concentration of antibiotic, allows the quantitative assessment of the MIC with the ease of a diffusion procedure.

While there is still no complete consensus of opinion on the treatment of intermediately and highly penicillin-resistant *S. pneumoniae* (4, 5, 7), the categorization of susceptibility is clearly important and should be considered along with the site of the infection and the patient's response to the chosen antibiotic. Most authorities would agree that central nervous system infections caused by highly resistant and intermediately resistant isolates cannot be treated with penicillin alone and that a modification of therapy is necessary. Until recently, broad-spectrum cephalosporins, such as cefotaxime or ceftriaxone, were used as alternative central nervous system infection antibiotics. Reports of elevated cefotaxime MICs coupled with clinical and microbiological treatment failures have made accurate susceptibility results necessary for a variety of antibiotics (2, 11, 19). Isolates of *S. pneumoniae* intermediately resistant to penicillin but resistant to cefotaxime or ceftriaxone are rare but may be becoming more prevalent (unpublished data).

Ngui-yen et al. (15) evaluated the use of the E test for penicillin susceptibility testing of 32 isolates of *S. pneumoniae*. Ninety-four percent agreement was found between E test results and the MIC reference method results. In a study
by Jorgensen et al. (8), there was 80.4% overall agreement between E test and NCCLS reference broth microdilution MICs for S. pneumoniae. Our study showed an overall agreement of 82.4%. All E test results, including the 19 discrepancies, were within one twofold dilution of the reference broth microdilution results.

The E test offers an alternative for the determination of antimicrobial susceptibility. Good growth of S. pneumoniae is achieved on the medium used, and the E test strips are easy to apply. Results are readily discernible and reproducible when the test is performed according to the manufacturer’s instructions. While there were differences between the two methods, all of the differences occurred at the breakpoints between susceptibility categories, traditionally problematic when one is comparing results based on a twofold dilution scheme. This difficulty is compounded by the fact that the E test provides results on a linear scale, so these results are not directly comparable to the twofold dilution results provided by microdilution in many cases. Also, the present proposed guidelines for the interpretation of susceptibility of broad-spectrum cephalosporins are based on sparse clinical data and may be too conservative (19). Nonetheless, given careful performance and awareness of possible errors, the E test appears to be a reliable method for the determination of the antimicrobial susceptibility of S. pneumoniae.

REFERENCES