Unreliability of Disc Diffusion Test for Screening for Reduced Penicillin Susceptibility in Neisseria meningitidis

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The 2-U penicillin and 1-μg oxacillin discs proposed for screening meningococci for susceptibility to penicillin were evaluated by using MICs measured by the E test. The discs yielded unacceptably high frequencies of misclassification of susceptibility category and should be abandoned in favor of MIC estimations. An agreed breakpoint for reduced penicillin susceptibility in meningococci is needed for the E test.

Penicillin susceptibility testing of meningococci has long been a difficult area, and authorities recommend MIC determination as the method of first choice (10). Nevertheless, the traditional disc diffusion test has remained in use as a practical and relatively inexpensive method. Campos and coworkers have argued that problems of interpretation encountered with regular 10-U penicillin discs may be reduced by using 2-U penicillin or 1-μg oxacillin discs (2, 3). These discs were introduced in Israel in 1992 and 1995, respectively, and since introduction of regular MIC measurements obtained by using the E test in 1995, sufficient strains have been examined to permit an adequate analysis of their value.

The E test has been shown in several studies to be an acceptably accurate method for determining MICs for Neisseria meningitidis (1, 4–6). Despite the E test’s high cost, the relatively small number of cases of meningococcal disease encountered in Israel each year makes it an attractively practical option for MIC estimations.

The present study was conducted from January 1995 through July 1997 with 133 consecutive clinical isolates of N. meningitidis submitted to the Israel National Center for Meningococci at Tel Hashomer. Laboratories isolating N. meningitidis from patients are required by law to submit the isolates to the Center for characterization. Internal audit has revealed that 95% of these N. meningitidis isolates are received at the Center, where they are serogrouped and serotyped, their antibiotic susceptibilities are tested, and they are stored at −70°C.

Disc diffusion testing was performed by using 2-U penicillin and 1-μg oxacillin discs (Sanofi Diagnostics Pasteur, Marnes La Coquette, France) according to National Committee for Clinical Laboratory Standards recommendations (7). Blood (5%) was added to the Mueller-Hinton agar. Data on record at the Israel National Center for Meningococci from previous years, obtained with 10-U penicillin discs, showed that Mueller-Hinton agar gave significantly larger zone diameters than Mueller-Hinton agar with 5% blood (MHB). A total of 278 strains examined with MH gave an average inhibition zone of 42.4 mm (median, 40 mm; standard deviation, 7.4), while 315 strains tested on MHB gave a mean of 35.6 mm (median, 35 mm; standard deviation, 4.7) (P < 0.0001 by the t test). Others have also found unsupplemented Mueller-Hinton media wanting, so various additions have been used, including blood (4, 8, 9). The definitions of reduced penicillin susceptibility in meningococci are:

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<thead>
<tr>
<th>Penicillin E Test MIC (µg/ml)</th>
<th>0.25</th>
<th>0.19</th>
<th>0.125</th>
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<tr>
<td>1</td>
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<td>3</td>
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<td>8</td>
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1μg Oxacillin disc zone diameter (mm)

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susceptibility used for the analyses were those proposed by Campos et al. (3): inhibition zone diameters of ≤26 mm around the 2-U penicillin disc and ≤10 mm around the 1-μg oxacillin disc.

The penicillin E test (AB Biodisk, Solna, Sweden) was performed according to the manufacturer’s instructions, on MHB. With few exceptions, tests were performed by the same individual. The conventional penicillin MIC of ≥0.1 μg/mL was used to denote strains with reduced susceptibility. This has limitations in respect to the E test, which are elaborated below.

Figures 1 and 2 indicate the relationships between disc diffusion zone diameters and MICs. The data show clearly that the 1-μg oxacillin disc was a poor predictor of penicillin susceptibility, since it is very close to the 0.1-μg/mL breakpoint, as shown in our data, an appreciable number of strains straddle the 0.094- to 0.125-μg/mL MIC range, suggesting that the question of an agreed E-test breakpoint for reduced penicillin susceptibility also needs to be further addressed if data from different centers are to be compared. (This study was presented in part at the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, 28 September to 1 October 1997.)

REFERENCES