Isolation of Moderately Penicillin-Susceptible Strains of Neisseria meningitidis in Argentina

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Four strains that were moderately susceptible to penicillin and/or ampicillin were found among 54 consecutive isolates of meningococci recovered from patients in one pediatric hospital in Argentina from October 1991 to December 1992. Disk diffusion tests performed with 2 U of penicillin failed to detect one strain. These findings suggest that attention should be paid to changes in the susceptibility patterns of meningococci in order to anticipate therapeutic failures in the future.

Moderately penicillin-susceptible meningococci (MIC between 0.12 and 1 μg/ml), have been reported, to our knowledge, only in the United Kingdom, South Africa, and Spain (1,5,9). Penicillin resistance in those cases was not due to β-lactamase production, as was previously described elsewhere for Neisseria meningitidis (4). These meningococci have altered forms of PBPs, probably as a result of the incorporation of genetic material from commensal Neisseria species (10).

In the present study we report the results of susceptibility tests with penicillin and ampicillin performed between October 1991 and December 1992 on meningococci isolated in the Hospital de Pediatría "Prof. Dr. J. P. Garrahan," Buenos Aires, Argentina.

Fifty-four consecutive isolates of N. meningitidis recovered from blood or cerebrospinal fluid samples from hospitalized patients were included in this study. The isolates were identified to the species level by standard bacteriological procedures. Serotyping was performed by agglutination using a commercial monospecific serum (Bacto Neisseria meningitidis Antiserum; Difco, Detroit, Mich.).

Dilution susceptibility tests were performed by the broth macrodilution method. HTM broth prepared in this laboratory was used in this study (6). This medium was selected instead of Mueller-Hinton broth because we find it easier to determine the end point with HTM broth. Moreover, both media were compared in penicillin broth macrodilution susceptibility tests using the same inoculum of 20 strains of N. meningitidis. Four strains showing growth in HTM broth at 24 h did not produce turbidity in Mueller-Hinton broth before 48 h of incubation. MICs obtained with both media did not differ in more than one dilution (data not shown). The final inoculum density was approximately 5 × 10^5 CFU/ml, and the tubes were incubated at 35°C for 18 h in a forced-air incubator, as was indicated by the National Committee for Clinical Laboratory Standards for other organisms (7).

Diffusion tests were carried out with Mueller-Hinton agar containing 5% sheep blood as was previously suggested (3). These plates were incubated at 35°C under humid conditions without increased CO2. The disks contained penicillin (2 U) and oxacillin (1 μg) (Laboratorios Britania, Buenos Aires, Argentina).

β-Lactamase production was tested by the nitrocefin method (Oxoid, Basingstoke, England) (8). Forty-six strains were serotyped with a monospecific serum (Difco). Thirty-five belonged to group B, three belonged to serogroup C, and eight were characterized as W-135. The rest were not viable at the time they were to be serotyped.

When dilution susceptibility tests were performed, three strains appeared moderately susceptible to penicillin (MICs = 0.125, 0.25, and 0.5 μg/ml). None of these three was a β-lactamase-positive meningococcus. Serogroups of resistant strains can be seen in Table 1. No conclusions can be drawn, because there were only three isolates classified as moderately penicillin-resistant strains. However, it should be noted that penicillin resistance is likely to be related to the C-2b serotype in Spain (2), and two of the three strains of N. meningitidis characterized as moderately susceptible to penicillin in the present study belonged to the W-135 serotype.

In 29 strains, correlation between results of dilution and diffusion tests could be established by using the breakpoints suggested by Campos et al. (3) (Fig. 1). Moderate sensitivity to penicillin (MIC ≥ 0.125 μg/ml) was not indicated in one strain by the diffusion method results. Three penicillin-susceptible (clearing zone diameter of ≥28 mm) but oxacillin-resistant (clearing zone diameter of <11 mm) strains were found by the diffusion method. Only one strain was characterized as moderately susceptible to ampicillin (MIC = 0.125 μg/ml) and susceptible to penicillin (MIC = 0.03 μg/ml), as previously described (3).

Some strains of N. meningitidis moderately susceptible to ampicillin and penicillin have now been isolated in Argentina. As J. Campos et al. suspected, by applying simpler detection methods, these strains could be isolated outside the United Kingdom, Spain, and South Africa (3). However, we did not find a good correlation among disk diffusion oxacillin resistance and broth dilution ampicillin resistance. Moreover, one strain failed to be recognized as a penicillin-resistant meningococcus by the disk diffusion test performed with disks containing 2 U of penicillin.

We do not know the significance of this kind of resistance. Patients infected with moderately penicillin-susceptible meningococci have been successfully treated with ceftriaxone in this hospital. We do not know what would have happened if these patients had been treated with penicillin. Ceftriaxone

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is used in this hospital for the initial treatment of meningitis in children aged 2 months to 5 years. In the four cases, this therapy was not changed to penicillin. In the literature, however, there are reports of patients successfully treated with high doses of penicillin (2).

Perhaps the greatest importance of these findings involves the attention that should be paid to changes in the behavior of meningococci regarding susceptibility to beta-lactams. It is possible that MICs for moderately susceptible strains of Neisseria meningitidis will increase, as occurred with Neisseria gonorrhoeae, where the MICs rose to levels 500- to 1,000-fold higher than those for penicillin-susceptible strains (9). We now have the advantage that we can monitor such events by performing diffusion tests routinely. This practice might prevent therapeutic failures in the future.

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REFERENCES

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TABLE 1. Distribution of different serogroups among penicillin- and ampicillin-resistant strains of N. meningitidis

<table>
<thead>
<tr>
<th>Pattern of resistance*</th>
<th>Serogroup (MIC [µg/ml])</th>
<th>(no. of strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp* (≤0.06) Pen* (≤0.06)</td>
<td>B (34), C (2), W135 (6), nontyped (8)</td>
<td></td>
</tr>
<tr>
<td>Amp* (&gt;0.06) Pen* (≤0.06)</td>
<td>B (1)</td>
<td></td>
</tr>
<tr>
<td>Amp* (&gt;0.06) Pen* (&gt;0.06)</td>
<td>W135 (2), C (1)</td>
<td></td>
</tr>
</tbody>
</table>

* Amp*, ampicillin susceptible; Amp*, moderately ampicillin susceptible; Pen*, penicillin susceptible; Pen*, moderately penicillin susceptible.

![MIC (µg/ml)](image)

FIG. 1. Comparison of penicillin susceptibility results performed by an HTM broth dilution method and an agar diffusion method using Mueller-Hinton broth containing 5% sheep blood