Spurious Ampicillin Resistance by Testing Haemophilus influenzae with Agar Containing Supplement C

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Ampicillin resistance (minimal inhibitory concentration ≥10 µg/ml) in the absence of beta-lactamase activity by Haemophilus influenzae was noted in tests performed with Mueller-Hinton agar containing one lot of supplement C. All strains, except five with known resistance due to beta-lactamase activity, were inhibited by 0.6 µg or less of ampicillin per ml of chlorozactolized blood agar.

Jorgensen and Jones (1) recently described a simplified medium for use in testing of Haemophilus influenzae susceptibility to ampicillin. This medium consists of Mueller-Hinton agar (MHA) or broth (MHB) with added supplement C, a sterile desiccated yeast concentrate which, according to its manufacturer (Difco Laboratories, Detroit, Mich.), contains glutamine, coenzyme (V factor), hematin (X factor), cocarboxylase, and other factors necessary for the cultivation of fastidious organisms. This medium is suitable for the growth of H. influenzae and retains the agar's light color and clarity.

According to Jorgensen and Jones (1), ampicillin-susceptible strains of H. influenzae were inhibited by concentrations of 1 µg or less of ampicillin per ml, whereas resistant strains required at least 8 µg/ml. These data corroborated an earlier report by Thornsberry and Kirven (4), who also later demonstrated a close relationship between resistance to ampicillin and beta-lactamase production (3).

Our laboratory has found clinical isolates of H. influenzae that require at least 5 µg of ampicillin per ml for inhibition in MHA containing supplement C, but lack demonstrable beta-lactamase by either acidimetric or iodometric techniques. This has prompted us to examine the new medium in more detail.

Twenty-five isolates of H. influenzae were tested against ampicillin, penicillin, cephalothin, cephalaxin, and chloramphenicol at concentrations of 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15, and 0.07 µg/ml. Also tested were five ampicillin-resistant strains, obtained through the courtesy of C. Thornsberry, Center for Disease Control, Atlanta, Ga. Each concentration of each antibiotic was mixed in parallel in MHA containing 10% supplement C (lot no. 606528) and MHA containing 1% IsoViteX (BBL, Cockeysville, Md.) and 2% hemoglobin solution (Difco), hereafter referred to as MHA-C and MHA-H, respectively.

The inocula were prepared by scraping growth from chocolate blood agar plates into MHB. The turbidity of each suspension was adjusted to match that of a one-half McFarland no. 1 standard. A 1:10 dilution of the adjusted suspension was then delivered onto the surfaces of the agar plates with an inocula replicating apparatus (2). The plates were then incubated at 35 C in an atmosphere with 10% CO₂ for 16 h. The minimal inhibitory concentration (MIC) was the lowest concentration without visible growth.

The results are shown in Table 1. With the exception of chloramphenicol, MIC values obtained in MHA-C exceeded those obtained in MHA-H. With strains fully susceptible (MIC ≤0.6 µg/ml) to ampicillin and penicillin in MHA-H, these differences were at least eight-fold and frequently at least 16-fold. Because none of these strains exhibited beta-lactamase activity by acidimetric or iodometric techniques, we presume that they were susceptible to ampicillin but would have been erroneously described as resistant if one had relied exclusively on the MIC values obtained in MHA-C.

The cause of the discrepancies in MIC values remains unexplained. Jorgensen and Jones (1) reported plate dilution MIC values of ampicillin tested against susceptible strains of H influenzae ranging between 0.0625 and 0.500 µg/ml, a range compatible with what we obtained in tests performed shortly after their initial report of the procedure (J. H. Jorgensen and P. M. Jones, Prog. Abstr. Intern. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 353, 1974). The results in this study were obtained with a new lot of supplement C, because we had used up the original lot and the manufacturer no longer had any supplement corresponding to that lot number. We have since tested the same strains used...
Table 1. Relationship of MIC in MHA-H and MHA-C

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Range in MHA-H (µg/ml)</th>
<th>Relationship of MIC in MHA-C (no. of strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-2</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.15 – &gt;10</td>
<td>4</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.07 – &gt;10</td>
<td>5</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0.3 – &gt;10</td>
<td>2</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>0.6 – 10</td>
<td>8</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.3 – 0.6</td>
<td>23</td>
</tr>
</tbody>
</table>

* Each antibiotic was tested against 30 strains.
* These data are shown as number of times less or greater than MIC in MHA-H.
* Denotes equivalence.
* Ampicillin-resistant strains with MIC ≥10 µg/ml in both media.

in this study against ampicillin in MHA containing other lots (no. 611437 and 612176) of supplement C and have obtained MIC values corresponding to those of MHA-H.

Although the addition of supplement C to MHA is a simple and convenient technique for susceptibility testing of H. influenzae in clinical laboratories, our results demonstrate the continuing need for quality control of antimicrobial susceptibility tests by concurrently testing known susceptible and resistant strains of H. influenzae and the utility of attempting to confirm apparent ampicillin resistance of H. influenzae by demonstration of beta-lactamase activity.

LITERATURE CITED