Comparison of Rapid Methods of Antimicrobial Susceptibility in *Haemophilus influenzae*

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**ABSTRACT**

A method is described for the rapid, simultaneous determination of the MIC’s of chloramphenicol and ampicillin to *Haemophilus influenzae*. Excellent agreement was observed between the Autobac method and the agar dilution method for antimicrobial susceptibility. All ampicillin resistant *Haemophilus* isolates produced beta lactamase and none of the susceptible strains produced this enzyme.

**Introduction**

*Haemophilus influenzae* is a leading cause of meningitis in children of the age group one to four years. Until recently the antibiotic of choice for the treatment of *H. influenzae* meningitis was ampicillin. However, several reports have now documented the growing resistance of this microorganism to ampicillin.\(^4\,6\,7\,9\) Consequently, the American Academy of Pediatrics has suggested that patients suspected of *H. influenzae* meningitis be treated with ampicillin and chloramphenicol. Tilton and Ryan\(^8\) recently published a method for the rapid determination of the minimum inhibitory concentration (MIC) of ampicillin using *H. influenzae* as the test organism. Predictably, the presence of chloramphenicol resistant *H. influenzae* has now been reported.\(^2\,3\) This paper presents a semi-automated procedure for the simultaneous rapid testing of ampicillin and chloramphenicol on clinical isolates of *H. influenzae*.

**Materials and Methods**

Thirty two isolates of *H. influenzae* type b representing both resistant and susceptible strains were tested. The isolates were maintained on chocolate agar slants and, prior to analysis, were transferred to chocolate agar plates, incubated in 8 percent CO\(_2\) for 16 hrs and the resultant growth used as an inoculum. These organisms were obtained from local hospitals as well as the Center for Disease Control (CDC).

Antibiotics were prepared as previously described\(^8\) except that chloramphenicol (USP) was dissolved in aqueous solution and elution discs made representing final concentration ranging from 0.75 to 25.0 mcg per ml.

Agar dilution susceptibility testing was performed on chocolate agar using methods specified by Ericcson and Sher­ris.\(^5\) The analysis for the enzyme beta lactamase was done according to the method of Thornsberry and Kirven.\(^11\)
AUTOBAC METHODOLOGY

Eugonic broth (Pfizer) was supplemented by the addition of Fildes reagent (BBL) to Eugonic broth. The 10 percent concentration of Fildes reagent to Eugonic broth was prepared by the addition of 2.0 ml of the reagent to 16.0 ml of Eugonic broth. Each batch of Fildes reagent was analyzed for excessive light scattering. If particles were present above the background of the Eugonic broth, the reagent was then filtered through a 0.22 micrometer membrane filter prior to use. The H. influenzae inoculum was prepared by the addition of growth from a chocolate agar plate to 5 ml of Autobac saline solution in an Autobac inoculum standardization tube. The concentration of the inoculum was standardized with the Autobac nephelometer to 1.5 to 3.0 \times 10^7 colony forming units per ml (cfu per ml). Two ml of this inoculum was added to 18 ml of the supplemented Eugonic broth. Ampicillin and chloramphenicol discs were added to the cuvet. The cuvet was filled as previously described and incubated at 36°C in the Autobac shaker.

READING OF THE AUTOBAC TEST

The MIC was determined by comparing growth in the control well of the cuvet to growth in the wells containing decreasing concentrations of ampicillin and chloramphenicol. The cuvet was read in "normal mode" of the Autobac. The MIC's were read as the lowest concentrations of ampicillin and chloramphenicol in which no growth of the organism occurred. The arbitrary dilution endpoint for the reaction can be described as follows:

<table>
<thead>
<tr>
<th>LSI</th>
<th>INTERPRETATION</th>
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<tbody>
<tr>
<td>0.0 - 0.50</td>
<td>R (growth)</td>
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<tr>
<td>0.50 - 1.00</td>
<td>S (no growth)</td>
</tr>
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Results

In figure 1 are compared the MIC results for ampicillin generated by the Autobac method and by agar dilution. There was exact methodological agreement in 30 of 32 isolates of H. influenzae. In only two instances was there a difference between methods and then only a one dilution difference was observed; in all cases agar dilution MIC's were higher than those observed by Autobac. Of the 32 isolates tested, 21 were resistant to ampicillin. In figure 2 are shown similar data for chloramphenicol. Two of the isolates were resistant to chloramphenicol. In 28
of 32 instances there was precise agreement between methods. As with ampicillin, the four isolates in which there was disagreement showed higher MIC's by agar dilution.

The beta lactamase test was performed on all isolates. Only those which were resistant to ampicillin (MIC > 2.0 mcg per ml) produced beta lactamase. In those with susceptible MIC's (< 2.0 mcg per ml), no enzymatic activity was detected.

Discussion

The resistance of *H. influenzae* to ampicillin is plasmid mediated and results in the formation of the enzyme beta lactamase. Numerous isolations of these resistant organisms have been reported throughout the world. Consequently, there has been increased reliance on second-line antibiotics such as chloramphenicol. Current therapeutic recommendations are that both ampicillin and chloramphenicol be initiated until the susceptibility of the *H. influenzae* isolate to ampicillin is known. Beta lactamase assays on isolated strains have been suggested and should be in the diagnostic regime of every clinical microbiology laboratory. There have been rare reports of *H. influenzae* resistant to ampicillin in which no beta lactamase could be detected. However, this should not discourage the routine use of this rapid and useful test.

Confirmation of resistance or susceptibility is now available in three hours by a rapid ampicillin MIC test. With the advent of chloramphenicol resistance in *H. influenzae*, it became imperative to test isolates rapidly for both ampicillin and chloramphenicol resistance. The Autobac methodology was modified to measure two MIC's (ampicillin and chloramphenicol) in the same 12 well cuvet in three hours.

Although the beta lactamase test determines ampicillin susceptibility, there is no comparable routine screening test for chloramphenicol resistance. Only three chloramphenicol resistant isolates have been identified; however, if such resistance is plasmid-borne, then these strains may be expected to increase in prevalence. Of the three isolates, only one was resistant to both ampicillin and chloramphenicol. It should be noted that there are 2 distinct populations of Haemophilus observed in figure 2. Although the Autobac method produced an MIC of 12.5 mcg per ml in both of the organisms found to be resistant by the agar dilution method (25 mcg per ml), the truly susceptible organisms had MIC's of 0.25 mcg per ml. If the Autobac method is used, then an equivocal MIC should be confirmed by agar dilution.

The Autobac, although originally designed, evaluated, and marketed for routine antimicrobial susceptibility testing has proved invaluable in producing rapid, accurate MIC data on clinical *H. influenzae* isolates, the treatment of which is markedly influenced by the now unpredictable antibiotic susceptibility patterns.

References