Letters to the Editor

Optochin sensitivity of β-haemolytic streptococci group A

Optochin (ethyl hydrocuprein hydrochloride) sensitivity testing is routinely performed by most laboratories for the differentiation of Streptococcus pneumoniae from other streptococci.

In our laboratory all streptococci, seen by Gram staining of blood cultures, are tested for sensitivity to optochin. The optochin test results are read after 4–6 h incubation at 36°C, along with other antibiotic sensitivity tests.

This procedure was followed on a set of blood cultures from a patient who was subsequently shown to have a β-haemolytic streptococcus group A infection; however, there was a zone size >14 mm round the optochin disc on the original chocolate agar plate. On overnight testing β-haemolysis was apparent, but the isolate still gave a zone >14 mm on a fresh chocolate agar plate.

We have investigated the phenomenon of optochin sensitive β-haemolytic streptococci group A and our results are presented.

Material and methods

One hundred strains of group A β-haemolytic streptococcus from various clinical sites were used. The isolates were each streaked on to three sets of chocolate agar (Oxoid) and Columbia 5% horse blood agar (Oxoid) plates. Two or three colonies of each group A β-haemolytic streptococcus were streaked on to one quarter of the test medium and a 6 mm optochin disc (Mast) was placed on the inoculum. The plates were then incubated at 36°C for 18–24 hours under varying conditions: (a) one set in a normal aerobic incubator, (b) one set in a 5% CO₂ incubator, and (c) one set in an anaerobic incubator (Don Whitley Scientific).

The original optochin sensitive group A β-haemolytic streptococcus and a fresh laboratory isolate of Streptococcus pneumoniae as a control were also included.

After incubation zones of inhibition were measured. A zone size >14 mm was recorded as sensitive. All zone sizes <14 mm were discounted. The table records the sensitivity of the 100 strains tested.

<table>
<thead>
<tr>
<th>No of strains</th>
<th>Room air Choc</th>
<th>Room air BA</th>
<th>5% CO₂ Choc</th>
<th>5% CO₂ BA</th>
<th>Anaerobic Choc</th>
<th>Anaerobic BA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>9</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Resistant</td>
<td>91</td>
<td>98</td>
<td>93</td>
<td>100</td>
<td>98</td>
<td>94</td>
</tr>
</tbody>
</table>

Choc = chocolate agar.
BA = blood agar.
Sensitive = zone of inhibition >14 mm diameter.
Resistant = zone of inhibition <14 mm diameter.

The zones of inhibition shown by group A β-haemolytic streptococci were comparable to that of the Streptococcus pneumoniae control. With the exception of blood agar in 5% CO₂, zones of inhibition were shown on both blood agar and chocolate agar under different atmospheric conditions.

Four strains were sensitive to optochin only on blood agar in anaerobic conditions. Another four strains showed sensitivity only on chocolate agar in aerobic conditions. Other strains were sensitive to optochin randomly on blood agar or chocolate agar or both in aerobic, CO₂, or anaerobic conditions.

There did not seem to be any correlation between optochin sensitivity and the type of medium used or the conditions of incubation.

None of the sensitive strains was bile soluble.

Discussion

Optochin sensitivity has been described for use in the identification of Streptococcus pneumoniae by several workers. Although reports of false sensitivity of the α-haemolytic streptococci do occur (<1%), there have been no such reports with β-haemolytic strains. The largest zone size suggested by Austrian—that is, 14 mm—was used to define a sensitive strain.

We have found that the incidence of β-haemolytic streptococci sensitive to optochin is much higher than that reported for α-haemolytic streptococci.

The effect of a CO₂ environment in reducing the zone size of some strains is also mimicked by Streptococcus pneumoniae. Ragsdale and Stanford described how the presence of 5% CO₂ diminished the zone size of Streptococcus pneumoniae to optochin and suggested that strains that do not grow in atmospheric conditions should be tested by the bile solubility technique.

We offer no explanation of the variation from strain to strain within the group A β-haemolytic streptococci and cannot suggest a mode of action for optochin. Kreger et al showed that electron dense agglomerates appeared in the cytoplasm of treated cells, but this was found to be a non-specific effect.

Although we do not believe that there are many laboratories that would mistake a β-haemolytic streptococcus group A for a Streptococcus pneumoniae on an overnight culture, on rapid 4–6 h testing the β-haemolysis may not be evident and another character, such as microscopical appearance, must also be used before presumptive identifications are made.

GH BURGESS
J KHOR
Department of Microbiology,
St James Hospital,
London SW12

References

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G H Burgess and J Khor

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