Simple method for detecting penicillinase-producing Neisseria gonorrhoeae and Staphylococcus aureus

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SUMMARY A filter paper acidometric test, using bromocresol purple as pH indicator, for detecting penicillinase-producing Neisseria gonorrhoeae (PPNG) and Staphylococcus aureus gave complete agreement with the chromogenic cephalosporin and rapid iodometric methods when performed on 300 strains of gonococci and 70 strains of Staph aureus. The test is cheap and simple and may be used to screen for penicillinase-producing strains of N gonorrhoeae and Staph aureus.

Introduction

A recent study1 of the detection of PPNG showed that the chromogenic cephalosporin, rapid iodometric (tube), and penicillin disc diffusion methods gave complete agreement with all the strains tested. A filter paper iodometric technique detected 99% of PPNG without any false-positive results and could be used to screen for such strains by laboratories needing to test many strains.

In this study a method adapted from Slack et al2 of a filter paper acidometric technique, using bromocresol purple as pH indicator, was evaluated. It was technically simpler than the filter paper iodometric method. The results of this test on 300 strains of gonococci and 70 strains of Staph aureus are reported.

Materials and methods

Freshly isolated N gonorrhoeae strains, obtained from clinical specimens, were isolated and identified as described.1 These isolates were tested for the production of penicillinase by the chromogenic cephalosporin, rapid iodometric (tube), and filter paper bromocresol purple (BCP) acidometric methods.

The Staph aureus strains were isolated from clinical specimens. They were identified by colonial morphology on blood agar plates, Gram staining, and the coagulase test. Penicillinase production was detected by the chromogenic cephalosporin and BCP acidometric tests.

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Filter paper BCP acidometric method

The penicillin solution consisted of 5% crystalline penicillin (buffer-free) and 0.2% bromocresol purple dissolved in 0.05 molar phosphate buffer, pH 8.0 (37.5 mg KH₂PO₄ and 842 mg Na₂HPO₄·2H₂O in 100 ml distilled water). The penicillin solution was divided into small aliquots and kept at −20°C. When an aliquot was in use it was kept at 4°C.

In the test a piece of Whatman No 1 filter paper measuring 5 × 6 cm was placed in a Petri dish. The penicillin solution was then dropped on to the paper to saturate it. With a bacteriological loop a number of colonies from a culture was spread over an area approximately 5 mm in diameter. Several different strains of gonococci may be tested on the same paper, separated from each other by about 1 cm. The paper was incubated at 37°C for 30 minutes with the Petri dish cover on. Yellow zones were formed by colonies producing penicillinase but not by the others. A similar procedure was followed with strains of Staph aureus except that results were read after 60 minutes incubation.

The chromogenic cephalosporin and rapid iodometric (tube) methods were carried out as described.1

Results

PPNG strains

A total of 150 PPNG and 150 non-PPNG strains were tested. There was complete agreement between the results of all three tests with all the strains tested (table). No difficulty occurred in distinguishing between penicillinase-positive and penicillinase-negative strains with the BCP acidometric method. For 148 of the penicillinase-positive strains, yellow zones could be detected within 10 minutes. Two of the strains required 20 minutes.
and chromogenic cephalosporin
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TABLE
Comparison of results of three methods for
detecting penicillinase-producing Neisseria gonorrhoeae
and Staphylococcus aureus

<table>
<thead>
<tr>
<th>Methods</th>
<th>Results for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N gonorrhoea</td>
</tr>
<tr>
<td>Chromogenic cephalosporin</td>
<td>+</td>
</tr>
<tr>
<td>Rapid iodometric</td>
<td>+</td>
</tr>
<tr>
<td>Acidometric (bromocresol purple)</td>
<td>+</td>
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</tbody>
</table>

+ Positive; − negative

STAPH AUREUS
Fifty of the strains of Staph aureus gave positive and
20 negative results with both the BCP acidometric and
chromogenic cephalosporin tests (table). For
most of the penicillinase-positive strains wide yellow
zones could be seen within 30 minutes' incubation.
However, for a few weak penicillinase-producers it
was necessary to incubate for 60 minutes; the results
for these were best seen by viewing through the
bottom of the Petri dish against indirect light.

The penicillin solution has been kept at −20°C
and 4°C for three months and one month respec-
tively without deterioration in the quality of the
reagent. On freezing the solution the colour turned
yellow but on thawing the purple colour was
restored. This did not adversely affect the reaction.

Discussion

The filter paper BCP acidometric method has several
favourable features which make it a convenient
method for the routine detection of PPNG. The
reagents for the test are cheap and easily obtained
and have a reasonably long shelf-life. The method is
simple and quick and several strains of gonococci
may be tested on the same piece of filter paper. The
end results are clear-cut. This study shows that the
method gives complete agreement with the chromo-
genic and rapid iodometric methods.

The technique has several advantages over the
filter paper iodometric test. There are fewer reagents
and their concentrations are less critical. The test also
requires fewer steps and the incubation time is more
flexible. With gonococci the results are normally read
after 30 minutes' incubation; this can be delayed for
60 minutes without adversely affecting the results.
The method can also be used to detect penicillinase-
producing Staph aureus but it is necessary to extend
the incubation period to 60 minutes.

The technique described differs from that of Slack
et al2 in several ways. By introducing a buffer and
decreasing the concentration of penicillin it is
possible to maintain the stability of the solution for a
longer period. The use of a larger piece of filter paper
makes it more convenient to screen a greater number
of gonococci. It should be pointed out that the
penicillin solution should not be left to dry on the
filter paper and kept. On rehydrating such reagent-
impregnated paper the migration of water across the
paper will cause the reagents to be carried along with
the water and be unevenly distributed; if the piece of
paper is small such migration is negligible.

References

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