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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 study[1] 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 al*[2] 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 (BPC) 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 KH_2PO_4 and 842 mg $Na_2HPO_4.2H_2O$ 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.

TABLE *Comparison of results of three methods for detecting penicillinase-producing Neisseria gonorrhoeae and Staphylococcus aureus*

Methods	Results for: *N gonorrhoeae* +	*N gonorrhoeae* −	*Staph aureus* +	*Staph aureus* −
Chromogenic cephalosporin	150	150	50	20
Rapid iodometric	150	150		
Acidometric (bromocresol purple)	150	150	50	20

+ 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 respectively 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 chromogenic 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 al*[2] 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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