Technical methods

A rapid paper-strip method for the detection of penicillinase production by penicillin resistant strains of *Staphylococcus aureus*

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The size and nature of the zone of inhibition round a benzylpenicillin disc have been widely used as guides to the sensitivity or resistance of a strain of *Staphylococcus aureus*. However, the diameter of the zone of inhibition depends on many factors, including inoculum size, depth of medium, and potency of the antibiotic test disc. Inoculum size, in particular, may be difficult to standardise when sensitivity testing is carried out directly from clinical material.

The demonstration of penicillinase production is a valuable guide to potential clinical resistance. Many methods for the detection of staphylococcal penicillinase have been described, but some are unsuitable for routine use because of their complexity.

The principles of the method which we describe here are as follows: Strains of *Staph. aureus* that are able to produce penicillinase are induced to do so by growth in a subinhibitory concentration of methicillin. The induced β lactamase hydrolyses added penicillin to form penicilloic acid, resulting in a fall of pH. A suitable indicator is used to demonstrate this pH change.

This acidimetric principle has been used previously in an agar overlay method (Wong and Soo-Hoo, 1976). In the present method, however, benzylpenicillin and an indicator are incorporated into paper strips. When desiccated and stored under optimum conditions the test strips retain their sensitivity for many months.

Material and methods

CHOICE OF MEDIUM AND INDICATOR

Tests are performed on culture plates similar to those used routinely for the determination of methicillin sensitivity of *Staph. aureus* isolates. Organisms capable of producing penicillinase do so in the area immediately surrounding the zone of inhibition associated with a methicillin disc. The highly buffered sensitivity test agars tend to obscure the fall in pH caused by the hydrolysis of added benzylpenicillin; a poorly buffered agar (2% Columbia, BBL) was found to give consistent and satisfactory results.

The pH of freshly poured columbia agar was found to be 7·2, but after surface inoculation with *Staph. aureus* and overnight incubation the pH rose to 8·2-8·6. An indicator which encompassed this latter range but which would register a fall of pH was required; cresol red (pH range 7·2-8·8) was found to be the most satisfactory.

PREPARATION OF TEST STRIPS

Soluble benzylpenicillin (unbuffered), 600 mg, is dissolved in 3 ml 0·1% aqueous cresol red; 0·1 ml N/10 sodium hydroxide is added. Three to four filter papers (Whatman, No. 1, 9 cm diam) are saturated with this solution. The impregnated papers are placed in the lids of 9 cm diameter Petri dishes and are dried in a vacuum desiccator. When dry, the papers are cut into strips measuring approximately 5 × 12 mm; these strips are placed in a screw-capped jar containing freshly activated silica gel and are stored in darkness at 4°C.

THE TEST

A columbia agar plate is inoculated with the test strain of *Staph. aureus*. A 10 μg methicillin disc is applied to the surface of the medium and the plate is incubated for 18 hours at 30°C. After incubation the diameter of the zone of inhibition is read. A prepared test strip is then placed radially across the edge of the zone of inhibition (Figure). It will rapidly assume a purple-red colour. In the positive test a yellow colour then develops at the zone edge while both ends of the test strip remain purple-red. In the negative test the yellow colour does not develop, and the entire strip remains purple-red. The test, therefore, acts as its own negative control, for the outer portion of the strip registers the pH of the lawn of uninduced organisms.

Positive results will generally become apparent within four minutes of the application of the test strip, although weak penicillinase producers may take 10 minutes to give a positive result. The results should be read within 40 minutes, since after that time the strips fade as indicator diffuses into the medium.

COMPARISON WITH REFERENCE METHODS

Eighty-nine strains of *Staph. aureus* isolated from clinical material were tested for penicillinase pro-

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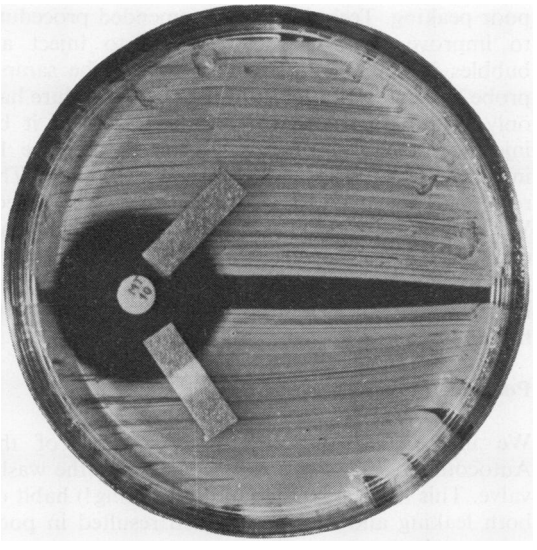

Figure *Methicillin sensitivity test plate with test strips for penicillinase applied. Upper: a non-penicillinase producing strain of* Staph. aureus. *Lower: a penicillinase producing strain.*

duction by the method under study, by the agar overlay method of Wong and Soo-Hoo (1976), and by a method using the chromogenic cephalosporin 87/312 (O'Callaghan *et al.*, 1972). This latter technique was modified as follows: 5 mg of the cephalosporin were dissolved by prolonged shaking in 10 ml 0·1 phosphate buffer at pH 7·0; 50 μl of the resulting solution were dispensed into wells of a microtitre tray. The test strains of *Staph. aureus* were incubated on columbia agar containing a subinhibitory concentration of methicillin to induce β lactamase. Colonies were picked and emulsified in phosphate buffer at pH 7·0. Of the resulting turbid suspension, 50 μl were added to the cephalosporin solution. A positive result, indicating the presence of β lactamase, was indicated by a red colour change.

The minimum inhibitory concentrations (MICs) of benzylpenicillin were determined by the multipoint inoculation of each strain onto columbia agar incorporating doubling dilutions of benzylpenicillin. The medium was contained in the wells of microtitre trays and not in Petri dishes to prevent the diffusion of induced β lactamase throughout the medium. An inoculum of approximately 10^3 colony forming units was used.

All strains were also tested for penicillin sensitivity by a disc diffusion method using 2% columbia agar and benzylpenicillin discs (1 unit). The inoculum of each test strain was adjusted to give a lawn of growth that was just confluent.

Results

Thirty-five strains gave positive results by all three methods for the detection of penicillinase and had MICs in the resistant range (0·25-16·0 mg/l). When tested by disc diffusion the diameters of the zones of inhibition ranged from 9 to 16 mm.

The other 54 strains gave negative results by all three methods for the detection of penicillinase production and had MICs in the sensitive range (0·016 0·064 mg/l). The zone diameters ranged from 26 to 34 mm when the strains were tested by disc diffusion.

Discussion

The complete agreement which we have found between the results given by the new method for the detection of staphylococcal penicillinase and by two reference methods indicates that the former may have an application in the routine laboratory, particularly when the sensitivity of a strain of *Staph. aureus* is in doubt. The determination of the sensitivity of a strain of *Staph. aureus* by disc diffusion methods is dependent on the inoculum size, whereas the detection of induced penicillinase is inoculum independent. For this reason the paper strip method for the detection of staphylococcal penicillinase may prove useful where the inoculum size is difficult to control, as when performing sensitivity tests directly from clinical material.

The method, although found by us to be useful for the detection of penicillinase production by strains of *Staph. aureus*, was unreliable when used in attempts to detect β-lactamase produced by *Haemophilus influenzae* and *Neisseria gonorrhoeae*. This was probably due to the greater buffering capacity of media required for the growth of these organisms.

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References

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