Technical method

Use of Intralactam for the early detection of staphylococcal β -lactamase

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The use of an acidimetric paper method for the detection of β -lactamase produced by *Haemophilus influenzae* and *Neisseria gonorrhoea* has been described (Slack *et al.*, 1977). β -lactamase producing strains of these organisms produce the enzyme in easily detectable amounts. β -lactamase producing strains of *Staphylococcus aureus*, however, produce little of the enzyme unless induced to do so by growth in subinhibitory concentrations of an inducing agent such as penicillin. Penicillinase-resistant semisynthetic penicillins, such as methicillin, are especially potent inducers of staphylococcal β -lactamase. Detection of this induced β -lactamase provides a guide to clinical resistance: methods have been described by Wong and Soo-Hoo (1976) and Wheldon and Slack (1978). However, these methods require a lengthy pre-incubation of the test organism in a subinhibitory concentration of methicillin.

In this study we have examined the ability of commercially prepared Intralactam to detect staphylococcal β -lactamase before and after induction of the enzyme.

Material and methods

A total of 442 strains of *Staph. aureus*, all recently isolated from clinical material, were tested for β -lactamase production using Intralactam Test Strips (Mast Laboratories, Derby Road, Bootle):

- (a) after growth on penicillin-free media;
- (b) after growth on a subinhibitory concentration of methicillin.

In addition, each strain was tested for β -lactamase production using the chromogenic cephalosporin (O'Callaghan *et al.*, 1972), nitrocefin, after induction in a subinhibitory concentration of methicillin.

Benzylpenicillin disc sensitivity tests were also carried out on each strain.

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β -LACTAMASE DETECTION IN NON-INDUCED STRAINS USING INTRALACTAM

Six colonies of each strain were picked and streaked across the test portion of an Intralactam strip which had been previously placed on a clean glass slide and moistened. The slide was then placed in a wet box in order to extend the time period of the test. A colour change from purple-grey to yellow was taken to indicate the presence of β -lactamase. In 208 strains, the time of this colour change was noted. Control uninoculated strips showed no loss of sensitivity throughout the period of the test.

β -LACTAMASE DETECTION IN INDUCED STRAINS

The strains were also grown in a subinhibitory concentration of methicillin to induce production of β -lactamase. This was carried out as follows: The strains were inoculated on to nutrient agar plates. A 10 μ g methicillin disc was applied to the surface of each plate. Plates were incubated overnight at 30°C. Colonies from the area immediately surrounding the zone of inhibition associated with the methicillin disc were tested for β -lactamase using nitrocefin as follows: 5 mg of nitrocefin were dissolved in 10 ml 0.1 phosphate buffer at pH 7.0. 50 μ l of the resulting solution were dispensed into the well of a microtitre tray. Several colonies of the induced strain were emulsified in the nitrocefin solution. A red colour change within 30 minutes was taken to indicate the presence of β -lactamase.

Each induced strain was also tested for β -lactamase production using Intralactam test strips. Several colonies of each induced strain were applied to a moistened Intralactam strip and results were read after 30 minutes.

PENICILLIN SENSITIVITY TESTING

The penicillin sensitivity of each strain was determined by the size and character of the zone surrounding a 1U benzylpenicillin disc, as in the method described by Stokes and Waterworth (1972).

Results

Of the 442 non-induced strains of *Staph. aureus*, 340 (77%) were found to produce β -lactamase when tested with Intralactam. After induction in a subinhibitory concentration of methicillin, β -lactamase production was confirmed in all these strains when tested with nitrocefin and Intralactam.

Fig. 1 β -lactamase production and benzylpenicillin zone size. Four series: a (82 strains), b (99 strains), c (190 strains), and d (81 strains). Strains found to be positive for β -lactamase by all three methods are shown hatched. Negative strains are represented by unhatched columns. One strain was positive with nitrocefin alone. Two strains in series b were positive only with Intralactam after induction. These three strains are represented by dotted lines.

Fig. 2 Time of appearance of positive results in 208 non-induced strains of known β -lactamase producing *Staph. aureus*.

Ninety-nine strains (22.4%) were shown to produce no detectable β -lactamase by these three methods. Two strains were found to produce detectable β -lactamase only when tested by Intralactam after induction. One strain gave a positive result with nitrocefin alone.

Correlation between the zone size on disc sensitivity testing and the detection of β -lactamase is shown in Figure 1. The disc sensitivities were

carried out in four separate series at different times and therefore have not been summated.

Accurate timing of the appearance of a positive result was carried out on testing of 208 strains using Intralactam without prior methicillin induction; these results are shown in Figure 2.

Discussion

Benzylpenicillin remains the drug of choice for the treatment of infections due to non β -lactamase producing strains of *Staph. aureus*; factors such as greater antibacterial activity, low serum binding, and greater tissue penetration favour the use of benzylpenicillin rather than flucloxacillin or cloxacillin. However, a minority of hospital isolates are penicillin sensitive; because of this fact cloxacillin (or flucloxacillin) is often advocated as appropriate treatment of staphylococcal infections before penicillin sensitivity has been determined. This may take up to 18 hours after isolation of the organism. In our timed series, β -lactamase was detected in 195 strains (94%) within an hour of primary isolation. Within 2 hours 203 strains (98%) were positive: at 4 hours this figure had risen to 207 strains (99.5%). The remaining strain gave a positive result after overnight incubation. The speed of this test allows β -lactamase detection to be carried out concurrently with the tube coagulase test and for results to be read simultaneously.

At present we are not sure whether the time of emergence of positive results using the method under study implies a quantitative estimation of β -lactamase activity.

The advantage of this method over earlier techniques necessitating induction of β -lactamase is the speed with which results are obtained.

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Letter to the Editor

Angioimmunoblastic lymphadenopathy and hypercalcaemia

We were interested to read the paper by Brearley *et al.* (1979) describing, in addition to the usual clinical features associated with angioimmunoblastic lymphadenopathy (AIL), the haematological and in some cases marrow abnormalities found in seven patients with this syndrome. As the spectrum of abnormal findings associated with this condition broadens we should like to report a recently encountered case in which a persistently raised serum calcium was found, as we are unaware of hypercalcaemia having been reported previously in cases of AIL.

A 27-year-old man presented with generalised hilar and para-aortic lymphadenopathy and fine pulmonary mottling. His initial serum calcium was in the normal range, but persistently raised levels in the order of 3.05 mmol/l (12 mg/100 ml) were soon recorded associated with a serum phosphorus of 0.8 mmol/l (2.5 mg/100 ml) and urinary calcium excretion of 31.0 mmol/24 h (1240 mg/24 h). Serum parathormone was 1.05 ng/ml (reference range up to 1 ng/ml with a normal serum calcium). This presentation initially suggested a diagnosis of sarcoidosis but axillary lymph node biopsy showed a diffuse alteration of nodal architecture by a mixed cellular proliferation of immunoblasts, plasma cells, lymphocytes, and some epithelial aggregates in association with an arborising vascular proliferation with endothelial hyperplasia and some periodic acid Schiff's positive material in vessels, lumens, and walls—the histological features of AIL as described by Lukes and Tindle (1975). Serum IgM, IgA, and IgG were all raised, and immunoperoxidase studies on the lymph node showed polyclonicity of the cellular infiltrate.

Initially, haematological investigations showed little abnormality but within two and a half months of the onset of the disease investigation revealed: haemoglobin 7.2 g/dl; leucocytes $3.8 \times 10^9/l$

($3800/mm^3$) with 31% lymphocytes and 16% eosinophils; platelets $17 \times 10^9/l$ ($1700/mm^3$); positive direct Coombs test; demonstrable rouleaux and cold agglutinins. Bone marrow aspiration was unsuccessful at two attempts. Treatment with steroids, transfusion, and cytotoxic drugs was ineffective, and the patient died three months after the onset of the disease from a klebsiella septicaemia. The serum calcium remained raised throughout this terminal phase of the disease.

Hypercalcaemia occurs in myelomatosis but is rare in lymphoma and leukaemia (Walker, 1974; Jayaraman and David, 1977). Evidence indicates that a bone-resorbing factor such as ectopic parathormone (Heath, 1976) or osteoclast-activating factor (Mundy *et al.*, 1974) is active in these diseases even in the presence of bony metastases. In this case with hypercalcaemia the serum parathormone is inappropriately high and is compatible with primary or ectopic parathormone secretion. Co-existent primary hyperparathyroidism seems unlikely in view of the initially normal serum calcium, its sudden increase, and absence of supportive radiological evidence. Since AIL may progress to immunoblastic sarcoma ectopic parathormone secretion is a probable hypercalcaemia-inducing mechanism. The release of osteoclast-activating factor may, however, be an additional mechanism since, as well as being demonstrated in patients with myelomatosis and lymphomas (Mundy *et al.*, 1974), it has been produced by phytohaemagglutinin stimulation of normal lymphocytes (Chen *et al.*, 1976). This observation may be especially pertinent since the whole histological appearance of angioimmunoblastic lymphadenopathy is that of a marked exaggeration of lymphocytic transformation.

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