Technical methods

Detection of beta lactamase activity of *Haemophilus influenzae*

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Strains of *Haemophilus influenzae*, type B, resistant to ampicillin are now being encountered in clinical practice in many different parts of the world, including the United Kingdom (Thornsberry *et al.*, 1976). Many of these strains have been associated with treatment failures. Recently, in America, the Center for Disease Control, the Medical Letter, and the American Academy of Pediatrics have warned of increased numbers of strains of *H. influenzae* resistant to ampicillin. In some institutions the frequency of these isolates appears to be increasing; three of 28 strains isolated from blood or cerebrospinal fluid over a 10-month period at a children's hospital in Boston, Massachusetts, were resistant to ampicillin (Smith, 1976). Furthermore, it appears that there may be an unusual propensity for a resistant strain to spread among childhood contacts (Schiffer *et al.*, 1974) and transferable resistance between strains has been demonstrated (Thorne and Farrar, 1975). Although reports of resistant strains are much less common in the United Kingdom, treatment failure of meningitis caused by ampicillin-resistant *H. influenzae* has been reported (Clymo and Harper, 1974). It would therefore seem prudent to perform sensitivity tests on all isolates of major clinical importance, such as those from meningitis, epiglottitis, cellulitis or osteomyelitis, by a reliable method, and in addition it is recommended that the prevalence of these strains and also of chloramphenicol-resistant strains be monitored on a regional and national basis (Cavanagh *et al.*, 1976). The method commonly used in Britain is based on antibiotic disc diffusion tests on chocolate agar using a disc content of 10 μg ampicillin, and, in our experience, the results are capricious. Results using a 2 μg disc are more reliable.

Initial therapy in children with proven or suspected severe infection due to *H. influenzae*, type B, such as sepsis, meningitis, epiglottitis, arthritis, osteomyelitis or cellulitis, must include an agent of probable efficacy. Administration of ampicillin and chloramphenicol has been recommended in areas where resistant strains have been well documented (American Academy of Pediatrics Committee on Infectious Diseases, 1975). No evidence of antagonism has been reported with meningitis due to *H. influenzae*, and antagonism could not be demonstrated *in vitro* with this organism (Ahronheim, 1975).

Strains of *H. influenzae* which are highly resistant to ampicillin are generally found to produce β lactamase. Only 1 out of 53 strains from all over the world was found not to produce β lactamase (Thornsberry *et al.*, 1976). Tests for β lactamase can therefore be used to detect this particular form of resistance. Various methods exist, such as the pH change with a phenol red indicator (Rosen *et al.*, 1972), the iodine starch assay (Foley and Perret, 1962), or, more recently, the chromogenic cephalosporin substrate assay (O'Callaghan *et al.*, 1972). All of these methods have been used successfully with *H. influenzae*. The use of the chromogenic cephalosporin produces very quick reliable results with a culture of *H. influenzae* but this substance is not yet used in most routine laboratories. We describe here the modification of a method used for penicillinase detection (Kjellander and Myrbäch, 1964) to test for β lactamase production in *H. influenzae*. This method is so simple that it can be performed easily in any diagnostic laboratory without special skills or materials. Results can be obtained within 6 to 8 hours of setting up the test. Speed is essential as the consequences of delayed effective therapy may be disastrous. Death has been reported as occurring within two days of hospital admission in infants suffering from meningitis due to ampicillin-resistant strains who were treated with this drug (Thomas *et al.*, 1974).

Method

A chocolate agar plate is swabbed or flooded with a culture of Oxford staphylococcus NCTC 6571 which will result in an almost confluent bacterial growth.

An ampicillin disc (10 μg) is placed in the centre of the plate and a heavy inoculum of the test strain is streaked radially from the disc outward in one direction and a control strain in the opposite direction to produce growth about 0·25 cm wide. The plate is then incubated with 5% CO_2 at 37°C

Received for publication 22 December 1976

for 18 hours. Fifty strains of *H. influenzae* were examined, including seven which were known to produce β lactamase when tested using the chromogenic cephalosporin test. Only these seven strains produced a positive result by our technique.

When the strains of *H. influenzae* produce β lactamase the staphylococcal and haemophilus growth meet at an acute angle, forming a peak towards the antibiotic disc, and the zone of inhibition appears heart-shaped. Several strains may be tested on the same plate. The results of testing two β lactamase producing strains and one fully sensitive (non β lactamase producing) strain are shown (Figure).

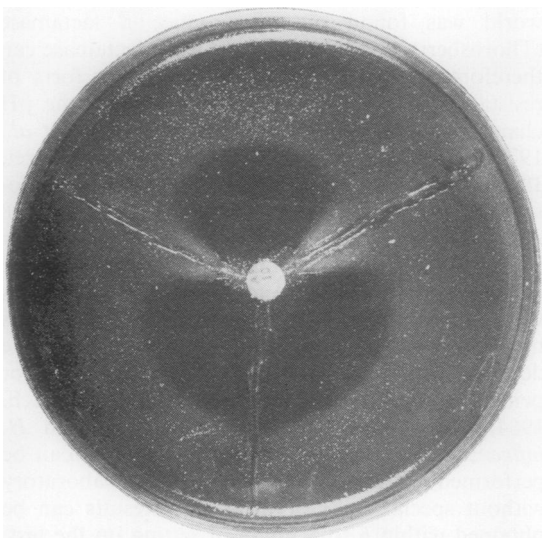

Figure *Two β lactamase producing and one non-producing strain of* H. influenzae *inoculated radially round a* 10 μg *ampicillin disc, the plate having previously been seeded with* Staph. aureus. *β lactamase producers give rise to deep indentations of growth in an otherwise circular zone of staphylococcal inhibition.*

Discussion

Although in some Gram-negative bacilli β lactamases are easily detectable only after disruption of large numbers of cells, this is not the case with *H. influenzae*. In this test sufficient β lactamase is available to diffuse away from the growth and cause the breakdown of ampicillin such that the seed layer of *Staphylococcus aureus* will grow where it would otherwise be inhibited.

The test described here offers a reliable method of testing for β lactamase production in *H. influenzae* which does not involve the use of specialised materials or techniques and is therefore suited for use in many routine diagnostic laboratories. Furthermore, routine use of this technique in selected laboratories could greatly facilitate regional monitoring of ampicillin resistance due to β lactamase production in clinical isolates of *H. influenzae*.

We thank Dr P. Cavanagh and Mr. P. B. Kilburn, of the Public Health Laboratory, Stoke, for help in supplying the cultures.

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A rapid blood lactate assay using a centrifugal analyser and 3-acetylpyridine-adenine dinucleotide

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It is being increasingly recognised that major forms of lactataemia and lactic acidosis are of clinical importance because they are amenable to treatment (Krebs *et al.*, 1975; Cohen and Woods, 1976). However, the diagnosis of lactic acidosis demands the routine availability of a robust, simple, and rapid lactate assay. That no such entirely satisfactory method at present exists is amply indicated by the ever increasing literature on lactate assays, each emphasising convenience, speed, and simplicity.

Of recent note is the demonstration that fluoride-stabilised plasma is a satisfactory specimen for lactate analyses (Westgard *et al.*, 1972), and that lactate determinations are possible with an electrochemical enzymatic sensor (Racine *et al.*, 1975; Durliat *et al.*, 1976), or with ferricyanide and lactate dehydrogenase from yeast (Durliat *et al.*, 1976), or by the conventional NAD and lactate dehydrogenase assay using reaction rate techniques on a centrifugal analyser (Pesce *et al.*, 1975) or miniature centrifugal analyser (Hadjiioannou *et al.*, 1976). The emphasis in each case is on convenience and speed. We describe a rapid, ratiometric, lactate assay, using a centrifugal analyser, based on the reaction involving 3-acetylpyridine-adenine dinucleotide (APAD), instead of NAD, which has been described by Holzer and Söling (1965) and Maurer and Poppendiek (1974). The advantage of this approach is that the APAD/APADH system has a more positive redox potential than the NAD/NADH system and therefore the product of oxidation, pyruvate, does not require trapping. The test formulation is therefore simpler.

Received for publication 11 January 1977

Material and methods

CHEMICALS

3-Acetylpyridine-adenine dinucleotide (APAD), supplied by P. L. Biochemicals Inc, Milwaukee, Wisconsin. Lot number 327001 (94% pure) was used throughout the entire project. A 13·5 mmol/l solution, in doubly distilled water, was prepared freshly each day.

Lactate dehydrogenase from rabbit muscle. Three formulations were used:

(1) Crystalline suspensions in 3·2 mol/l ammonium sulphate suspension having a specific activity of about 500 U/mg protein supplied with the Lactate Test Combinations by BMC Diagnostics/Biochemicals Ltd, St. Laurent, Quebec. Stable for many years at 4°C.
(2) Lyophilised, salt-free powder having a specific activity of about 400 U/mg protein supplied by Sigma Chemical Co, St. Louis, Missouri. 5 mg was dissolved in 6·8 mol/l D-glycerol. Stable for one month at 4°C.
(3) Same lyophilised material described above dissolved in doubly distilled water. Prepared freshly each day.

Glycine-EDTA buffer 1 mol/l glycine containing 5·37 mmol/l disodium EDTA. pH adjusted with 1 mol/l NaOH. Stable for one week at 4°C.

Working buffer mixture 1 ml of the lactate dehydrogenase preparation was added to 12 ml of the glycine-EDTA buffer.

Working lactate standard L-(+)-lactate, lithium salt, supplied by ICN Nutritional Biochemicals, Cleveland, Ohio. 6 mmol/l solutions were prepared with doubly distilled water. Aliquots were frozen at −20°C and were found to be stable for at least two months.

ENZYME ASSAY

Lactate dehydrogenase activity was assayed by the method of Tuckerman and Henderson (1973) at 37°C.