Technical method

An evaluation of Intralactam, a preparation for the detection of β-lactamase production by *Haemophilus influenzae*

D. B. WHELDON AND MARY P. E. SLACK *Department of Bacteriology and Regional Public Health Laboratory, The Radcliffe Infirmary, Oxford, UK*

Haemophilus influenzae type b is a frequent cause of meningitis and other dangerous childhood infections. Ampicillin has been regarded by some as the drug of choice, particularly in the United States, after reports of chloramphenicol toxicity. However, ampicillin-resistant strains of *H. influenzae* are now widely distributed and form a variable proportion of all strains isolated. Howard (1977) found an overall incidence of ampicillin resistance in *H. influenzae* of 1·5% in England and Scotland, while in the United States Smith (1976) found that of 28 strains of *H. influenzae* isolated from blood or cerebrospinal fluid, three were resistant to ampicillin. Ampicillin resistance in *H. influenzae* is almost always due to β-lactamase production; intrinsic resistance, although recognised, is uncommon (Thornsberry *et al.*, 1976).

Disk sensitivity testing by conventional methods gives highly inoculum-dependant results. Additionally, *H. influenzae* is a relatively slow-growing organism, and results may take some time to appear.

Detection of β-lactamase must provide the most rapid means of inferring ampicillin resistance. Many methods are available for detection of this enzyme, but few have found their way into the routine laboratory. The plate method of Kjellander and Myrbäch (1964) is commonly used and gives reliable results but needs several hours' incubation before results are obtainable. Methods using the chromogenic cephalosporin (O'Callaghan *et al.*, 1972) and the paper strip acidimetric method (Slack *et al.*, 1977) probably represent the most rapid and convenient methods available at the present time.

Intralactam (Mast Laboratories, Bootle, Merseyside, UK) is a commercial preparation of the acidimetric paper strip method. Each test paper consists of a strip of filter paper impregnated with penicillin and bromocresol purple. The product is presented in a sealed aluminium container, which includes 25 strips and an active desiccant. The strips have a shelf-life of one year when stored at 4°C, although in our experience they remain active for longer, provided the strips are kept in their container. The strips must be kept dry, or a slow hydrolysis of the penicillin component will occur, resulting in an irreversible colour change to yellow.

In this study the reliability and ease of use of Intralactam were evaluated. Results obtained with this new product were compared with those obtained utilising the chromogenic cephalosporin substrate, Nitrocefin (Compound 87/312, Glaxo Research Ltd, Greenford, Middlesex, UK). In addition, minimum inhibitory concentrations of ampicillin for some of the test strains of *H. influenzae* were determined.

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Material and methods

A total of 131 strains of *H. influenzae*, all isolated from clinical material, were tested for β-lactamase production using the detection methods under study.

INTRALACTAM

A test paper strip was placed on a clean glass microscope slide and moistened with distilled water with a Pasteur pipette. Sufficient water was added to make the strip visibly damp but not saturated. Care should be taken to use neutral distilled water; we have found that application of water with a low pH may of itself turn the whole strip a yellow colour. A bacteriological loop was used to pick six colonies of a plate culture of a test strain of *H. influenzae* and to apply this growth across a portion of the paper. Known positive and negative controls were also applied to the appropriate portions of the test paper (Fig. 1). A positive result, shown by the colour change from purple-grey to yellow 10 minutes after application, was deemed to denote β-lactamase production. No colour change was seen when non-β-lactamase producing strains were tested.

NITROCEFIN (CHROMOGENIC CEPHALOSPORIN SUBSTRATE, 87/312)

A working solution of Nitrocefin was prepared as follows: 0·5 ml diethyl sulphoxide was added to 5 mg of the powdered cephalosporin. On dissolution of the solid, 9·5 ml 0·1 M phosphate buffered saline (pH 7·0) were added.

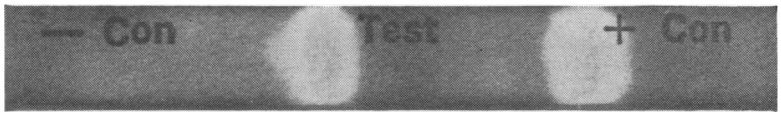

Fig. 1 *The Intralactam test strip. Appearance 10 minutes after application of test and control strains.*

Six colonies of each test strain of *H. influenzae* were emulsified in 0·5 ml of saline; 50 μl of this cell suspension were added to 50 μl of the working solution of Nitrocefin. The presence of β-lactamase production was shown by a colour change from yellow to red.

MINIMUM INHIBITORY CONCENTRATIONS OF AMPICILLIN

Minimum inhibitory concentrations (MICs) of ampicillin for 110 of the 131 strains of *H. influenzae* were determined by multipoint inoculation of chocolate agar incorporating doubling dilutions of ampicillin. An inoculum of approximately 10^5 colony-forming units was used.

Results

Of the 131 strains of *H. influenzae* tested for β-lactamase production, 26 gave positive results. No discrepancy was found in the results given by either method. Both methods were easy to carry out, and both gave clearcut results.

Of the 110 strains for which MICs had been carried out, 85 had an MIC of 0·5 μg/ml or less; all these strains were negative when tested for β-lactamase. Twenty-two strains had an MIC of 2 or more μg ampicillin/ml; 21 of these were positive when tested for β-lactamase. Three strains, all having an MIC of 1 μg ampicillin/ml, gave heterogenous results when tested for β-lactamase production. One gave a positive result, while the remaining two produced no demonstrable β-lactamase. One strain, with an MIC of 16 μg ampicillin/ml, produced no detectable β-lactamase and was deemed to be intrinsically resistant to the antibiotic. These results are shown in Figure 2.

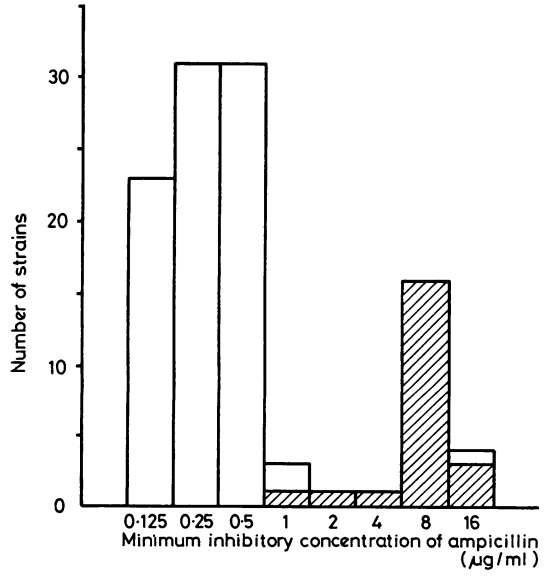

Fig. 2 *Sensitivity of 110 strains of* H. influenzae *to ampicillin.*

Discussion

Intralactam test papers were found in this study to provide a rapid and reliable means of detecting β-lactamase produced by *H. influenzae*. The test required small numbers of organisms and could be carried out on young cultures where growth was just visible to the naked eye.

However, we believe that conventional disk sensitivity testing should be carried out in order to detect the rare intrinsic resistance of a non-β-lactamase producing strain.

The principal advantage of Intralactam is that the test strips may be used directly from the shelf. There is no necessity for the preparation of fresh reagents as the test strips have a long shelf-life.

The reliability of Intralactam in the detection of *Haemophilus* sp β-lactamase should not be taken as a recommendation for its use in testing other Gram-negative organisms. We find that neither Intralactam nor Nitrocefin reliably identifies β-lactamase production by members of the Enterobacteriaceae. Excessively mucoid strains of *Escherichia coli*, *Klebsiella*, and *Enterobacter* spp frequently give false-negative results. We have not tested strains of *Neisseria gonorrhoeae* for β-lactamase production using Intralactam. There is, however, evidence that an acidimetric paper strip method may be used to detect gonococcal β-lactamase (Slack *et al.*, 1977).

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Requests for reprints to: Dr D. B. Wheldon, Bacteriology Department, The Radcliffe Infirmary, Oxford OX2 6HE, UK.

Letters to the Editor

Pneumococcal type 4 typing sera cross-react with type 2 pneumococcus

In 1977, a pneumococcus, which had been isolated in 1959 from a child with meningitis in Melbourne, showed a positive capsular reaction (Quellung) test with both type 2 and type 4 pneumococcal sera, Statens Seruminstitut, Copenhagen. A recently (1978) isolated strain from a New Guinean child in Port Moresby, also with meningitis, gave identical reactions. Positive results were obtained by counter-current immunoelectrophoresis with both sera. Further investigations with known type 2 pneumococci, including the reference strain NCTC 7466 (National Collection of Type Cultures, Colindale, UK), yielded similar results. Because pneumococci of types 1 to 5 inclusive have single identifiable capsular antigens, such a cross-reaction was unexpected (Mörch, 1943). Quantitative capsular tests were, therefore, done using our standard type 2 (Smith) and type 4 (Leal) strains with results as follows:

Pneumococcal		*Serum*	*Serum dilutions*				
Serotype	*Strain*		*Neat*	*1/5*	*1/10*	*1/20*	*1/40*
2	Smith	type 2	+	+	+	+	+
2	Smith	type 4	+	0	0	0	0
4	Leal	type 2	0	0	0	0	0
4	Leal	type 4	+	+	+	+	0

Three batches of type 4 serum were tested (comprising lot 1-78 and two earlier batches received in 1973 and 1975) with similar results. Our findings suggested that the type 4 sera contained type 2 antibody in low titre. This was confirmed by adsorption of the sera with a type 2 pneumococcus (NCTC 7466); the sera then gave negative results in the capsular reaction with the type 2 pneumococcus (but produced a positive result, as before, with the type 4 strain). The pneumococci from the children with meningitis each gave a titre of 40 with type 2 serum and failed to react with adsorbed type 4 serum; these strains could therefore be identified as type 2.

In January 1979, we received from the Statens Seruminstitut a new batch of type 4 serum (lot 2-78), which does not cross-react with type 2 pneumococcus. Many laboratories, however, may be using earlier batches of type 4 serum. Because capsular typing is usually done with neat (undiluted) sera, confusion could occur. Consequently, if an apparent cross-reaction occurs, the test should be repeated using serial dilutions of the typing sera with suitable controls.

I thank Mr Arthur Hewstone, Royal Children's Hospital, Melbourne, and Mr Michael Gratten, Port Moresby General Hospital, for supplying pneumococci, and Miss Carol Nicholson, Mr Andrew Moore, and Mrs Sandra Duncan for technical assistance.

Dr Jørgen Henrichsen, The Pneumococcus Laboratory, Statens Seruminstitut, Copenhagen, kindly verified our findings and supplied us with the new batch of type 4 pneumococcus serum.

DAVID HANSMAN
Department of Microbiology,
Adelaide Children's Hospital,
North Adelaide,
South Australia 5006

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