

SCREENING FOR BETA LACTAMASE PRODUCTION BY NEISSERIA GONORRHOEAE IN A SMALL LABORATORY

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Summary

Several methods of tests for beta lactamase production in *Neisseria gonorrhoeae* were compared to determine a suitable method for routine use in a small laboratory in a developing country. The test should be reliable, economical, easy to perform and easy to interpret using reagents which are easily available. A modification of the phenol red acidimetric method appears to satisfy these criteria.

INTRODUCTION

In 1976 several reports of beta-lactamase producing gonococci were made both in the United Kingdom¹ and the United States^{2,3}. Since then these beta lactamase producing strains have been reported in many countries including Japan, Korea, Philippines and Singapore⁴. The main objective of this project is to determine the most suitable method for rapid screening of beta lactamase in the context of a small laboratory in a developing country. This method should satisfy several criteria. It should be reliable and easy to perform. It should be economical. It should be easy to interpret and should not involve any sophisticated instruments.

MATERIALS AND METHODS

Fifty-nine specimens of *Neisseria gonorrhoeae* were screened for beta lactamase production. 36 non-beta lactamase producing strains and 2 known beta lactamase strains of gonococci were obtained from the Venereal Disease Reference Laboratory, Whitechapel, London and 13 non-beta lactamase producing and 8 known beta lactamase producing strains were obtained from St. Thomas's Hospital, London. All the strains of gonococci were screened for beta lactamase production using 5 methods namely chromogenic cephalosporin⁵, phenol red acidimetric method⁶, iodometric method⁷, microbiological assay and commercial **Intra-lactam** strips (Mast Laboratories) which is a modification of an acidimetric method performed on paper strips⁹. The chromogenic cephalosporin method, phenol red acidimetric method and iodometric method were performed as described below:

(a) Chromogenic Cephalosporin method:
5mg of chromogenic cephalosporin was dissolved in 0.5 ml of dimethylsulphoxide

and mixed with 9, 5 ml of 0.1M phosphate buffer at pH 7.0. This constituted the indicator solution.

(b) Phenol red method:

The indicator solution was prepared by adding 4.5 ml of sterile distilled water and 0.5 ml of 0.5% phenol red solution to a vial of one million units of unbuffered sodium benzyl penicillin (Crystapen, Glaxo Lab Ltd). Sodium hydroxide (1M) was added using a sterile straight wire until the solution just turned violet.

(c) Iodometric method:

The indicator solution consisted of seven parts of penicillin solution (10,000 U per ml), two parts of starch solution (10 gm per litre) and one part of iodine solution (2.03 gm of iodine and 53.2 gm of potassium iodide in 100 ml of water).

Several colonies of the test organism were picked off the plate and emulsified in a drop of sterile normal saline on a clean glass slide. A capillary tube was then dipped into the indicator solution and about one centimetre of the indicator solution was drawn into the tube by capillary action. Similarly about one centimetre of the bacterial suspension was drawn into the tube. There was usually spontaneous good mixing of the indicator solution and the bacterial suspension. Mixing could be facilitated by gently agitating the capillary tube. Both ends of the capillary tube were sealed using plasticine. The capillary tube was laid horizontally on a white surface and any colour change noted. The tests were read to be positive for beta-lactamase production if the indicator solution turned from yellow to red in the chromogenic cephalosporin method, from violet to yellow in the phenol red method and from blue to colourless in the iodometric method.

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TABLE 1
DETECTION OF BETA LACTAMASE PRODUCTION IN 59 SPECIMENS OF NEISSERIA
GONORRHOEAE USING DIFFERENT METHODS

METHOD	BETA LACTAMASE PRODUCTION	
	No. of strains POSITIVE	No. of strains NEGATIVE
Chromogenic cephalosporin	10	49
Phenol red method	10	49
Iodometric method	10	49
Microbiological assay	9	50
Intralactam strips	10	49

The microbiological assay was performed exactly as described in a previous paper⁸. The Intralactam test was performed following the manufacturer's instructions.

RESULTS

The chromogenic cephalosporin method was used as the **reference** method. The results are summarised in Table 1.

The **phenol** red method, iodometric method and the Intralactam strips gave perfect correlation with the chromogenic cephalosporin method. The microbiological assay gave one false negative result.

DISCUSSION

The chromogenic cephalosporin method for detection of beta lactamase is highly specific because the colour change occurs as a result of the opening of the beta lactam ring of the cephalosporin by beta lactamase. It is generally accepted as the most sensitive technique as well¹. It was an easy test to perform and the result easy to interpret. The indicator solution once prepared is stable for a fortnight if kept in a dark bottle. Unfortunately, the reagent itself is not yet available commercially and limited amounts can be made available only upon request to **Glaxo** Research Ltd.

The phenol red acidimetric method gave results that compared very well with **chromogenic** cephalosporin. The test was easy to perform and the colour change distinct. The reagents used were inexpensive and readily available. The phenol red indicator solution was found to be stable for up to fourteen

days if kept in the freezer compartment of a refrigerator.

The iodometric method also gave reliable results. The test was easy to perform and to interpret. The reagents involved were easily available. The major drawback of this method is the necessity of preparing the **penicillin**-buffer solution daily. Furthermore, spontaneous decolourisation of the indicator solution tend to occur after 30 minutes.

The microbiological assay for beta lactamase production was found to be less sensitive than other methods. It is more cumbersome to perform than the other methods described. The result could only be obtained after 24 hours incubation.

Intralactam strips gave reliable results. It was a very convenient method of beta lactamase testing. Its only drawback is its cost. In Malaysia it costs more than one Malaysian dollar per strip.

The various aspects of the tests are summarized in Table 2.

CONCLUSION

Of the various tests, the phenol red **acidimetric** method as modified in this paper appears to be the most suitable for beta lactamase detection in *Neisseria gonorrhoeae* in a small laboratory. It satisfies the various criteria of reliability, inexpensiveness, ease in performance and interpretation of the test and easy availability of reagents. This project was undertaken at the Hammersmith Hospital, London as part of the author's MSc thesis.

TABLE 2
COMPARISON OF THE VARIOUS METHODS OF TESTING FOR BETA
LACTAMASE PRODUCTION

TEST	Chromogenic cephalosporin	Phenol red	Iodometric method	Microbiological assay	Intra-lactam
Easy to perform	t	t	t	—	t
Easy to interpret	t	t	t	—	t
Rapid result	t	t	t	—	t
Inexpensive	—	+	t	t	—
Stability of reagents	t	t	t	t	t
Reagents easily available	—	+	t	t	—

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