A SIMPLE INEXPENSIVE METHOD FOR THE DETECTION OF B-LACTAMASE IN MICRO-ORGANISMS

Pages with reference to book, From 170 To 171

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Abstract

This describes a simple reliable and reproducible method for detecting B.lactamase production in micro-organisms. It involves impregnating starch paper in a solution of penicillin and placing two or three colonies of organisms to be tested, incubated for 30 minutes and flooded with iodine. The test is completed in 30 minutes which is helpful in the management of a patient (JPMA 37 : 1 70, 1987).

INTRODUCTION

Discovery of Penicillin in 1929[1] laid the foundation of antibiotics and their uses in therapy.
B lactamase existed in nature even before Penicillin was commercially available[2]. Since then a number of organisms which were sensitive to penicillin emerged as resistan: included in this group are B lactama se producing Staphylococcus, Haemo -philus, Neisseria, and various gram negative rods. Various commercial methods are available for detecting B.lactamases,[3] but they are expensive. We report here a modification of the iodometric technique[4] which could replace the detection of Blactamase producers within the reach of any small clinical bacteriology laboratory. The procedure is inexpensive, rapid and reliable.

MATERIAL AND METHODS

STARCH PAPER

Any good white paper e.g. 'Offset Paper', available locally is tested for the presence of starch, satisfactory samples turn blue-black on the addition of a drop of iodine solution. Starch positive white paper was cut into strips of 250 x 70 mm strips to fit the bottom of standard 850 mm petri dishes.

BENZYL PENICILLIN

Benzyl penicillin 100,000 units was mixed in 6.6 ml of phosphate borate saline (PBS) pH 7.2.

IODINE SOLUTION

Routine gram's iodine solution diluted to 1:3 was used.

ORGANISMS USED

Routine isolates of Escherichia coli, Pseudomonas, Kiebsiella pneumoniae, Staphylococcus aureus, Proteus, Enterobacter sp., Haemo-philus, Streptococcus were tested along with known B-lactamase positive organisms of Staphylococcus and Staphylococcus Oxford as negative control were used. The organisms were identified by routine identification methods including API strips.

PROCEDURE

The strips of starch paper were soaked in solution of penicillin containing 15000 units/cc (6.6 solution was sufficient for 30 strips), the soaked strips kept in a plastic container stored at 4°C and used as needed. The penicillin soaked strip was spread in a petri dish ensuring that no air bubbles are present. Each paper served for testing six organisms in addition to known (Bhactamase positive and negative controls. With the help of bacteriological loop, colonies of bacteria to be tested were picked up, transferred to the surface of the test paper, and spread on the paper so as to cover an area of

approximately 2-3 mm diameter. Each inoculum was placed at least 1.5 cm apart. The plates were mcubated at 35-36 C for 30 minutes. After incubation, the paper was flooded with iodine solution which was drained off immediately or alternatively two drops of solution was dropped on each inoculum, and results read after 30 seconds. Iodine causes the paper to turn uniformly black within 30 seconds, subsequently any areas where penicillin is inactivated are decolorized and contrast with the remaining part which is blue-black. This is because the starch in the 'offset' paper combines with iodine to form blue-black starch-iodine complex and where B-lactamase is present, it results in the enzymatic degradation of the B-lactamase ring of penicillin with the production of penicilloic acid which reacts with iodine causing dissociation of the starch iodine complex. The results must be read within 5 minutes after which the background tends to decolorize, making interpretation less clear. Clear cut and reliable results are obtained with high concentration of penicillin; concentrations of lower than 100 000 units result in weak reactions.

RESULTS

All the 100 stains of Staphylococcus aureus, E. coli, Salmonella, Kiebsiella, Pseudomonas, Enterobacter, Haemophilus and A citinobacter gave consistent results which were confirmed by resistance t6 10 ug penicillin discs.

DISCUSSION

The method is reproducible and efficient, the cost being almost negligible and within the reach of any small laboratory. It also enables the laboratory to confirm the true sensitivity of organisms isolated and eliminates the possibility of using expired sensitivity discs or discs of low standard. The method can be adapted and routinely used in all diagnostic laboratories.

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