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Detection of Metallo- β-lactamase Production in *Pseudomonas aeruginosa* by Various Phenotypic Methods.

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ABSTRACT

100 *Pseudomonas aeruginosa* species of various clinical samples are collected from central laboratory of a tertiary care hospital in south India. All the isolates are screened for carbapenem resistance by disc diffusion method using imipenem(10 µg). Isolates that were positive for carbapenem resistance were further subjected to metallo- β-lactamase screening by various phenotypic methods such as Modified Hodge test, Combined disc diffusion test and E-test. Varying results were obtained, however E-test results were satisfactory and comparable with standard strains.

Keywords: *Pseudomonas aeruginosa*, Carbapenems, Imipenem, Modified Hodge test, Combined Disc diffusion test, E-test.

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Introduction

Pseudomonas aeruginosa is a common Gram-negative bacilli associated with hospital infections and is often difficult to treat due to its multi/pan drug resistant drug profile. P. aeruginosa resistant to carbapenem is being increasingly reported [1]. Resistance to carbapenems is often mediated by production of Metallo-Beta-Lactamase (MBL) [2]. Infection with the metallo-beta-lactamase (MBL) producing organisms is associated with higher rates of mortality, morbidity, and health care costs. MBL producing Pseudomonas aeruginosa was first reported in Japan in 1991 and since then has been found in various parts of the world

MATERIALS AND METHODS

A total of 100 isolates of P. *aeruginosa* were cultured from clinical samples. The organism was identified by routine biochemical tests. The susceptibility to various classes of antimicrobials was determined by disc diffusion method in accordance CLSI guidelines [3]

Modified Hodge test (MHT)

The carbapenamase production is screened by Modified Hodge test [4]. A muller-Hinton agar was inoculated with a standard suspension (0.5 McFarland standard) of E.coli ATCC 25922.After drying the plate, a meropenem disc (10 µg) was placed on the center of the plate. The test strain was inoculated from the edge of the disc as straight line of about 2-2.5 cm long. The plate was incubated at 37°C for 18-24 hours. The plate was observed for identification of growth around the test streak(at the intersection of test strain and zone of inhibition).Enhanced growth is considered as positive for carbapenamase production (Fig 1)

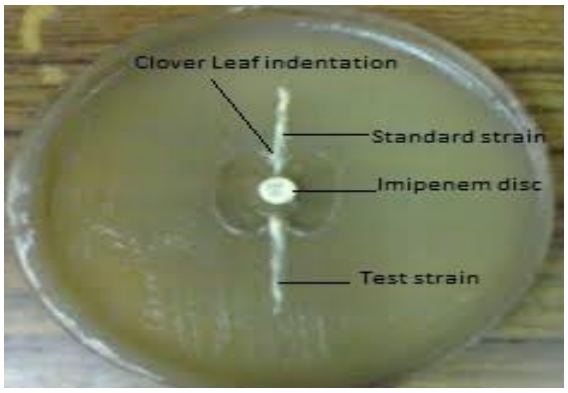

Figure 1: Modified Hodge test showing clover-leaf type indentation at the intersection of the test strain and the standard strain

Combined Disc Test (CDT)

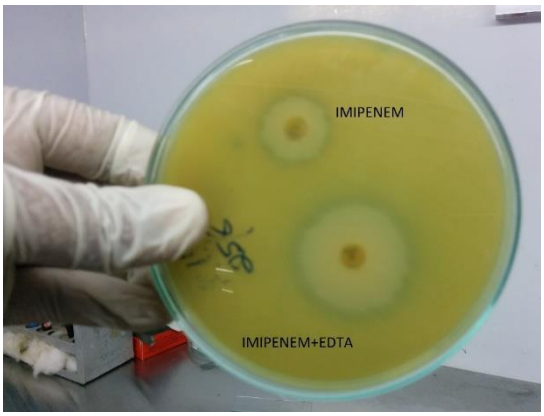

Figure 2: Imipenem-ethylenediaminetetraacetic double disc diffusion test Showing difference in the inhibition zones between the two discs is >7 mm Represents metallobetalactamase production

The MBL production is screened by combined disc diffusion test [5].The test strain (equivalent to 0.5 McFarland standard) was inoculated on the surface of MHA plate. On this two discs, one containing imipenem and the other with imipenem plus EDTA (Himedia) was placed on the surface of MHA. The plate was incubated at 37°C 18-24 hours. An increase in zone diameter of ≥ 7 mm around the imipenem- EDTA when compared to the meropenem disc alone is considered to be positive for MBL production (Fig 2)

E-test (EZY MICTM Strips Himedia-India)

The E-test MBL strip consists of double-sided dilution range of IM in one side and IM-EDTA dilution on the other side. The E-test strip determines the minimum inhibitory concentration (MIC) of the antimicrobial agent. The individual colonies of strain were suspended in liquid broth to attain a turbidity matching to 0.5 McFarland.With sterile cotton swab a lawn culture was made in the same way as for disc diffusion. Then E-test strip was placed on the agar with a sterile applicator.Then plate was incubated for 16-18 h at 37°C and results of MIC of IM and IM-EDTA read directly from the strip. Ratio of IM/IM-EDTA ≥8, presence of the phantom zone, and distortion of ellipse were interpreted as Positive results. This test was taken as the gold standard for detection of a MBL producer (Fig 3)

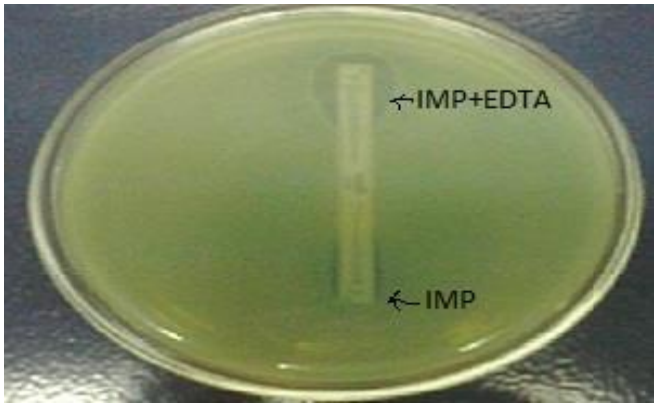

Figure 3: Metallobetalactamase (MBL) E-test showing minimum Inhibitory concentration ratio of imipenem (IM)/IM-EDTA of 8 i.e. MBL producing strain of Pseudomonas aeruginosa

RESULTS

Of the 100 isolates of P. aeruginosa, 24 showed resistance to imipenem by the disc diffusion method. A total of 18 isolates were positive for MHT. Furthermore, 17 of these 24 isolates exhibited a significant zone size enhancement with the EDTA impregnated discs when compared with the plain antibiotic discs. Of these, only 14 isolates were positive for Ezy- MIC test (the ratio of the value obtained for Imipenem (IPM): the value of Imipenem + EDTA (IPM+EDTA) was more than 8). The ATCC 27853 P.aeruginosa as expected did not exhibit a zone size enhancement.

Table 1

Meropenem/imipenem Resistant isolates	Detection of Carbapenamase Production			
	MHT Method (%)	CDT Method (%)	E-test Method (%)	Negative by all the method
24	75 (n=18)	70.8 (n=17)	58.3(14)	25(6)

DISCUSSION

Of the 24 carbapenem resistant strains studied MHT was positive in 18 strains and CDT was positive in 17 strains and E-test was positive in 14 strains. Though the results are varied, the differences are narrow. The CLSI has not recommended any standardized phenotypic methods for screening MBL in clinical isolates. Despite the good performance of inhibitor-based methods for the detection of MBL by using EDTA, it is not a specific test. False positive results have been reported in P. aeruginosa as EDTA acts on the membrane of the bacterial cell and increases the cell permeability.

Among the available phenotypic methods, E-test results were more promising and comparable with standard MBL positive strains.E-test was taken as the gold standard for detection of a MBL producer Since MBL mediated resistance accounts for 80 % of strains, it is crucial to develop a simple and rapid method to screen the MBL strains for a critically ill patients.

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