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INCIDENCE OF METALLO-BETALACTAMASE PRODUCING *PSEUDOMONAS AERUGINOSA* IN KESAR SAL MEDICAL COLLEGE AND HOSPITAL, AHMEDABAD

Awari Abhijit [1]* and Nighute Sunita[2]

* [1]Dept. of Microbiology, People's College of Medical Science & Research Centre, Bhopal – 462037
[2]Department of Physiology, Kesar SAL Medical College & Research Institute, Ahmedabad, Gujarat
***Corresponding Author:** abhijit.awari@yahoo.com

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ABSTRACT

Metallo-betalactamase producing pseudomonas aeruginosa have been reported to show resistance to carbepenem drugs. Detection of metallo-betalactamases producing pseudomonas is now important to prevent their spread as dissemination of these bacteria could be fatal to the patients.

The present study was undertaken over period of six months from November-2010 to May-2011 in Kesar SAL Medical College & Research institute, Ahmedabad to study the incidence of MBL producing pseudomonas aeruginosa from various clinical specimens. 100 isolates were obtained from different clinical samples of patients. These isolates were subjected to susceptibility testing to antipseudomonal drugs as per CLSI guidelines they were further screened for production of MBL by two methods i.e. EDTA impregnated imipenem double disc synergy method and Imipenem – EDTA combined disc test..

Out of hundred isolates 55 imipenem resistant isolates were screened for MBL production. 38 isolates showed MBL production. MBL production was found to be 9.09%. This presents therapeutic challenge to the clinicians and also needs proper selection of antibiotics especially carbapenems.

Keywords: Metallo-beta lactamase; *Pseudomonas*

1. INTRODUCTION

The carbapenems have been drug of choice for treatment of infections caused by gram negative bacilli Infections [1]. *Pseudomonas* shows resistance to carbapenems due to decrease outer membrane permeability, increased efflux system, alteration penicillin-binding proteins and carbapenem hydrolyzing enzymes *carbapenemase*[2]. The emergence of MBLS in pseudomonas species is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher cephalosporins[3]. Carbapenems available for use in India are imipenem and meropenem[4]. As treatment options are either not available or very expensive and may be toxic with poor outcomes[3].

2. MATERIALS AND METHODS

The study was conducted in the department of microbiology Kesar SAL Medical College & Research Institute from November-2009 to April-2011. Total no. of hundred isolates of *pseudomonas aeruginosa* obtained from various samples collected with universal safety precautions admitted to hospital. Samples were processed and identified by standard laboratory technique[5]. Routine antibiotic disc sensitivity testing was done with Ampicillin, Gentamicin, Amikacin, Ceftazidime, Cefepime, Cephotaxime, Ciprofloxacin, Chloramphenicol, Cefuroxime, Cefpodoxime, Norfloxacin, Nitrofurantoin, Tobramycin, Cephalexin, Cephazolin, Sparfloxacin, Pipercillin, Tazobactum, Colistin. All strains were found to be resistant to multiple drugs were tested for imipenem susceptibility.

Specimens processed were blood, urine, pus, wound swab, body fluids. Antibiotic susceptibility was performed on MH agar plates by Kirby baur's disc diffusion method. The results were recorded and interpreted as

per CLSI guidelines. *Pseudomonas aeruginosa* ATCC 27853 was used as a negative control. Imipenem resistance isolates were further screened for MBL production. [6'7'8'9]

1. Imipenem EDTA double disc synergy test. The test organisms were inoculated on the plates of Muller Hinton agar as recommended by CLSI (Clinical Laboratory standard institute) an imipenem (10 Microgram) disc was placed 20 mm centre to centre from blank disc containing 10 micro litters of 0.5 Mg EDTA (750 microgram). Enhancement of the zone of inhibition in the area between imipenem and the EDTA discs in comparison with zone of inhibition on the far side of the drug was interpreted as positive.
2. Imipenem EDTA combined disc test. Test organisms were inoculated on to the plates with Muller Hinton agar as recommended by CLSI. Two 10 microgram imipenem discs were placed on the plates and appropriate amount of 10 micro liter of EDTA solution were added to one of them to obtain the desired concentration of 750 microgram. The zone of inhibition was compared after 16-18 hours of incubation at 35°c. In combined disc test if the increase in inhibition zone was more than 7 mm than the imipenem disc alone was considered as MBL positive.

Preparation of 0.5 m EDTA solution 186.1g of disodium EDTA was dissolved in 1000 ml of distilled water and ph adjusted to 8 by using Noah. The mixture is sterilized by autoclaving.

3. RESULTS

Out of 100 isolates of *pseudomonas aeruginosa* 55 were imipenem resistant isolates were screened for MBL production 20 were isolated from pus, 12 from urine, 10 from blood and, 5 from vaginal swab, 2 from sputum, 3 from ear discharge, 2 from ICD catheter, 1 from wound swab.(Table-1)

Table-1

Samples	Isolates Imipenem resistant (*Pseudomonas aeruginosa*)
Pus	20
Urine	12
Blood	10
Vaginal Swab	5
Sputum	2
Ear Discharge	3
ICD Catheter	2
Wound Swab	1

Out of 55 imipenem resistant isolates tested for MBL production 38 exhibited more than 7 mm zone size by combined disc method and all 30 gave positive result by DDST method. (Table-2)

Table-2

Test	Total Isolates tested (n =55)	
	DDST Test	IMP-EDTA Combined disc Test
ATCC 27859 *Pseudomonas aeruginosa*	Negative	Negative
Isolates	30(54.54%)	38(69.09%)

Table-3 Antibiotic susceptibility testing showing resistant to various drugs

Antibiotic	Resistance (%)
Ampicillin	92 %
Gentamicin	84.7%
Amikacin	96.6%
Ceftazidime	96%
Cefipime	90%
Cephotaxime	100%
Ciprofloxacin	75%
Chloramphenicol	93%
Cefurroxime	70%
Cefpodoxime	95%
Norfloxacin	100%
Nitrofurantion	100%

Cephalexin	100%
Cephazolin	100%
Pipercillin	85%
Tazobactum	85%
Imipenem	100%
Tobramycin	98%
Sparfloxacin	85%
Polymyxin B	5%
Colistin	25%

4. DISCUSSION

Pseudomonas aeruginosa producing MBL was first reported from Japan in 1991, since then have been described from various parts of the world including Asia, Europe, Australia, South America, and North America, [10]

In this study the imipenem resistance in *pseudomonas aeruginosa* was found to be 55% compare to 26% by A. Varaiya, 69% by Behera etal, 59.52% by S.Irfan. 30% by Gupta etal and only 8.05% by Agrawal etal [11, 12, 13]

A study conducted by Mary etal, reported 42% MBL production by Pseudomonas aeruginosa.[14]

In another study Sarkar etal reported 54.54% MBL production by Pseudomonas aeruginosa.

In our study by different methods 69.9% (38 out of 55 isolates) were found to be MBL producers. The remaining negative isolates may have other mechanism of resistance such as impermeability of outer membrane or active efflux mechanism. In our study we employed two methods for MBL detection. The incidence of MBL producing pseudomonas by DDST method was found to be 30 out of 55 (54.54%) and by combined disc method the MBL producers were 38 out of 55(69.09%). The combined disc method was found to be more sensitive over DDST method in MBL detection.[4]

For MIC detection of imipenem the E test strip is recommended were one half of the strip is impregnated with an imipenem gradient across seven dilutions and other half with another imipenem gradient over laid with a constant concentration by EDTA.[15]

E test strip were not used in this study as they were very expensive.

CONCLUSION

Our study suggests that polymyxin B or colistin represent the best treatment options for MBL producing pseudomonas aeruginosa.

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