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INCIDENCE OF METALLO-BETA-LACTAMASE PRODUCING PSEUDOMONAS, ACINETOBACTER & ENTEROBACTERIAL ISOLATES IN HOSPITALISED PATIENTS

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ABSTRACT

Introduction: *The introduction of carbapenems like imipenem and meropenems into clinical practice represents a great advance especially for treatment of serious bacterial infections caused by beta lactam resistant bacteria. They are considered as a last resort most of the times for treatment of critically ill patients in intensive care units (ICUs) and high risk wards.* ***Aims and Objectives:*** *Hence the present study aims to find out the incidence of metallo-beta lactamase (MBL) production in Pseudomonas, Acinetobacter and Enterobacterial isolates from hospitalized patients.* ***Materials and Methods:*** *Isolates of Pseudomonas, Acinetobacter and members of Enterobacteriaceae family were identified by standard microbiological methods and antibiotic sensitivity was carried out by using Kirby Baur Disk Diffusion method as per CLSI guidelines. MBL production in imipenem resistant isolates was detected by using two methods i.e Imipenem-EDTA Double Disk Synergy Test (DDST) and Imipenem-EDTA Combined Disk Diffusion Test (CDDT).* ***Results:*** *289 isolates were included in the study, out of which 47 (16.2%) showed resistance to imipenem. Of these 47 strains 16 were P.aeruginosa, 16 were A.baumannii, 11 were E.coli, 2 were K.pneumoniae, and 1 was E. aerogenes and C.freundii each. 31(65.9%) of the imipenem resistant isolates tested positive for MBL production by both double disk synergy test (DDST) and combined disk diffusion test (CDDT).* ***Conclusion:*** *The detection of MBL producing isolates is of crucial importance and in the present scenario early detection will go a long way in making adjustments in empirical antimicrobial therapy and probably help a great deal in bringing down mortality rates for patients infected with MBL producing strains.*

KEY WORDS

Metallo-b-lactamase (MBL), Imipenem-EDTA double disc synergy test (DDST), Imipenem-EDTA combined disc test (CDDT), P. aeruginosa, A. baumanii

INTRODUCTION

The control of hospital-acquired infection caused by multi-drug resistant gram-negative bacilli has proved to be a particular problem over the last 20 years. Members of the family Enterobacteriaceae are among the most important bacterial human pathogens accounting for the majority of bacteria isolated from clinical samples.[1] That these Gram negative bacilli (GNB's) are rapidly acquiring resistance to one or more antimicrobial agents traditionally used for treatment is a matter of concern. Till now, extended spectrum beta-lactamase (ESBL) production by Gram negative bacilli was considered as the most important threat to clinical therapeutics. [2, 3]

The introduction of carbapenems into clinical practice represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria. The carbapenems available for use in India are imipenem and meropenem.[4] However, there

have been reports of resistance to carbapenems.[5,6] Carbapenem Resistance due to the production of metallo-beta-lactamases (MBL) in Gram-negative organisms is an increasing international public health problem.[7,8] The problem of MBL producing strains was originally confined to *Pseudomonas* and *Acinetobacter*. However, carbapenem resistance has been observed in members of Enterobacteriaceae family due to spread of MBL genes. The occurrence of an MBL positive isolate in a hospital environment poses not only a therapeutic problem, but is also a serious concern for infection control management.[9] Hence the present study aims at studying the incidence of MBL production in *Pseudomonas, Acinetobacter* & isolates of Enterobacteriaceae by two methods. 1) Imipenem-EDTA Double Disc Synergy Test (DDST) [10] & 2) Imipenem-EDTA Combined Disc Diffusion Test (CDDT). [13]

MATERIALS AND METHODS

Various isolates of Pseudomonas, Acinetobacter and Enterobacteriaceae obtained from different clinical samples like blood, pus, sputum, endotracheal secretions, catheter tips, urine, cerebrospinal fluid and various body fluids (synovial, ascitic, pleural) and identified by standard microbiological procedures were included in the study.

The isolates were tested for sensitivity to imipenem (10µg) using Kirby-Bauer method as recommended by National Committee for Clinical Laboratory Standards (NCCLS, now known as Clinical and Laboratory Standards Institute, CLSI).[9] Isolates of Pseudomonas, Acinetobacter and Enterobacteriaceae from various samples showing zone of inhibition less than 13mm were included in the study for MBL production by the following methods.

Imipenem-EDTA Double Disc Synergy Test (DDST):

The Imipenem-EDTA Double Disk Synergy Test was performed as described by Lee et al [10]. A 0.5 M EDTA solution was prepared by dissolving 186.1g of disodium EDTA· $2H_20$ in 1,000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilised by autoclaving. [11]

Direct colony suspension of test organism adjusted to match 0.5 McFarland turbidity was prepared and inoculated onto Mueller-Hinton agar plate as recommended by the National Committee for Clinical Laboratory Standards.[12]

An imipenem (10 µg) disc was placed 20 mm centre to centre from a blank disc containing 10 µl of 0.5 M EDTA (750µg). The inhibition zones of the imipenem and EDTA discs were compared after 16 to 18 hrs of incubation at 37^0C.

Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result. [10]

Imipenem-EDTA Combined Disc Diffusion Test (CDDT):

The Imipenem-EDTA Combined Disc Diffusion Test was performed by Yong et al.[13] Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by CLSI.[9]

Two 10µg imipenem discs (Becton Dickinson) were placed on the plate, and appropriate amounts of 10µl of EDTA solution was added to one of them to obtain the desired concentration (750µg).

The inhibition zones of the imipenem and imipenem-EDTA discs were compared after 16 to 18 hours of incubation at 37^0C. In the Combined Disc Test, the increase in inhibition zone with the Imipenem and EDTA disc was $\geq$ 7 mm than the Imipenem disc alone, it was considered as MBL positive. [13]

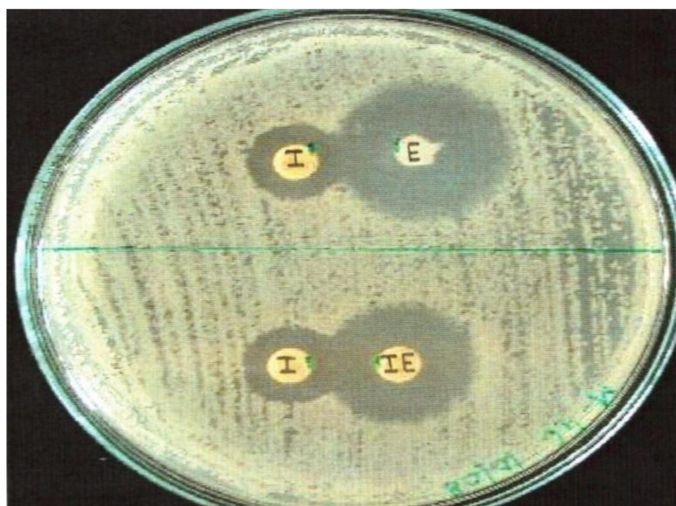
OBSERVATIONS AND RESULTS

Out of the 289 isolates included in the study 76 were Pseudomonas aeruginosa, 68 Acinetobacter baumannii, 65 E.coli, 48 Klebsiella pneumonia, 24 Enterobacter aerogenes and 8 were Citrobacter freundii.

Of these 289 isolates, 47 (16.2%) showed resistance to imipenem and were therefore further tested for MBL production. 31 of these isolates showed positive results for Metallo beta lactamase production by both DDST and CDDT methods as shown in **Table 1**.

Table 1: Total number of organisms isolated from different clinical samples of IPD patients, number of Imipenem resistant and MBL Producers in them.

Organism Isolated	Total No.	Imipenem R (%)	MBL Positive by DDST (%)	MBL Positive by CDDT (%)
P.aeruginosa	76	16 (21%)	8 (50%)	8 (50%)
A.baumannii	68	16 (23%)	9 (56%)	9 (56%)
E.coli	65	11 (16%)	10 (91%)	10 (91%)
K.pneumoniae	48	2 (4%)	2 (100%)	2 (100%)
E.aerogenes	24	1 (4%)	1 (100%)	1 (100%)
C.freudii	8	1 (12%)	1 (100%)	1 (100%)
TOTAL	289	47	31	31



Photograph 1: Isolated imipenem resistant strains showing positive test for MBL by both the methods DDST & CDDT.

DISCUSSION AND CONCLUSION

MBLs have been identified from clinical isolates worldwide, with an increasing frequency over the past few years and strains producing these enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections, as reported by Senda K et al. [15]

In our study about 21% of P.aeruginosa strains showed resistance to Imipenem of which about 50% were detected as MBL producers which is much higher than that obtained in a study conducted by Navneeth et al who reported 12% of MBL mediated Imipenem resistance in P.aeruginosa.[16] However the incidence of P.aeruginosa has been reported to be 10-50%

from various clinical specimens across the country.[17] In a study carried out by R Hasan et al in a tertiary care hospital in India, MBL production in Imipenem resistant isolates of Acinetobacter was 96.6% which is very high as compared to our study which showed 56% MBL production in Imipenem resistant Acinetobacter.[18] Since there is no standard guideline for detection of MBL, different studies have reported the use of different methods. Our study included the use of Imipenem- EDTA DDST and Imipenem-EDTA CDDT for detection of such isolates. Both the methodologies showed positive MBL results for the same 47 (Imipenem resistant) strains. The above methods were simple to perform and the materials used were cheap, non-toxic and easily accessible, making it highly applicable to routine clinical laboratories. It was seen that both the methods Imipenem-DDST and Imipenem-CDDT were equally effective for MBL screening. In our opinion, the detection of MBL producing isolates is of crucial importance not only in institutes with high prevalence of such isolates but also in those in which the phonotype of resistance have never been detected as this will go a long way in helping to make adjustments in empirical antimicrobial therapy and probably, the reduction of mortality rates for patients infected with MBL- producing strains.

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