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Occurrence of Metallo-Beta-Lactamase among Gram Negative Bacilli in a Tertiary Care Hospital.

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Abstract

Introduction: Metallo- beta lactamase is produced by many species of Gram negative bacteria and some species of Gram positive bacteria that are responsible for bacterial resistance in broad range of beta lactamase antibiotics including carbepenem family.

Materials and Methods: A cross sectional study was carried out in the Department of Microbiology Santosh Hospital and Ghaziabad. 150 Gram negative bacteria isolated from various clinical samples from both IPD and OPD patients were included in this study.

Results: The most common isolates were found to be E.coli 75(50%), Klebsiella species31(21%), Pseudomonas aeruginosa15(10%),Citrobacter species12 (08%),Acinetobacter species11(07.33%)and Proteus species 06 (04%), 30 imipenem resistant were isolates, 19(63.33%) were found to be positive for MBL production by Combined Disk Test while 11(36.66%) were positive for MBL production by Double Disk Synergy Test. Highest MBL production was found to be in *Pseudomonas aeruginosa* (26.66%).

Conclusion: Combined Disc Synergy Test(CDST) was found to be a better test as compared to Double Disc Synergy Test (DDST) as it is devoid of visual misinterpretation. A regular screening and monitoring system should be set up to prevent the dissemination of these genes in the country.

Introduction

The spread of multi drug resistant Gram negative bacilli in hospital setting is now seen as a globalized threat in majority of hospitalized patients especially those in the ICUs. Metallo- beta lactamase is an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics including the carbapenems. Since its first description in 2008 from a Klebsiella pneumoniae strain isolated from a patient repatriated to Sweden after hospitalization in New Delhi India, NDM-positive strains have been causing healthcare-associated outbreak worldwide; 24 NDM variants have been identified in various bacterial species responsible for healthcare-associated infection from the Enterobacteriaceae family and from Acinetobacter species and Pseudomonas species[1].

They compromise the activity of wide spectrum antibiotics creating major therapeutic difficulties with significant impact on the outcome of patient, appropriate antimicrobial selection, surveillance system and effective infection control procedure. New Delhi

metallo beta lactamse was first reported in *Klebsiella pneumoniae* and *E.Coli* isolate from a Swedish, 59 year old patient from India who was previously admitted to hospital in New Delhi[2]. In India the prevalence of metallo-beta lactamase ranges from (7.5-71%) [3-4].but there are very few documented reports in India from burns and surgical wards. The aim of the study was to determined the occurrence of metallo-beta lactamase among the Gram negative bacterial isolates obtained from various clinical samples received in the bacteriology laboratory.

Materials and Methods

The study was conducted in the Department of Microbiology , Santosh Medical College and Hospital, Ghaziabad for a period of one year from October 2017 to 2018. The aim of the study was to isolate the gram negative bacilli from the clinical samples and determine the occurrence of MBL positive isolates.

Methodology: All specimens including urine, pus, blood, sputum, stool,and body fluids such as cerebrospinal fluid and pleural fluid, received in the bacteriology lab were processed according to standard bacteriological procedures.

Screening of Metallo-Beta Lactamase

The isolates which showed resistance to Imipenem i.e (zone ≤ 19 mm) were screened as Metallo- beta lactamase positive strains[5].

Confirmation of Metallo-Beta Lactamase

Confirmation of Metallo-beta lactamase production was done by using two tests which included combined disc test (CDT) using imipenem and EDTA (CDT-IMP) and double disc synergy test (DDST) using imipenem disc and simple disc with EDTA (DDST-IMP) as described by Yan et al.[6] and Behera et al.[7] In combined disk test (CDT), if zone of inhibition of IMP-EDTA disk was ≥ 7 mm that of IMP disk alone, it was considerd as MBL positive[8].

Statistical test: Chi -square test was used to detect statistically significant correlation among variable.Significance defined as 95% ($P < 0.05$).

Results: Of the 150 Gram negative isolates were isolated from various clinical samples. Bacteriological profile showed that 75 (50%) isolates were *E.coli* which predominated the population followed by 31 (21%), *Klebsiella species*, 15 (10%)*Pseudomonas aeruginosa*, 12(08%) *Citrobacter species*, 11(7.33%) *Acinetobacter species* and 06(04%) *Proteus species.*

Double- disk synergy test (DDST) using imipenem and EDTA with simple disc produced large zone of inhibition toward the imipenem+EDTA disk shown in(figure no-1) Pseudomonas aeruginosa ATCC (27853) was used as control.

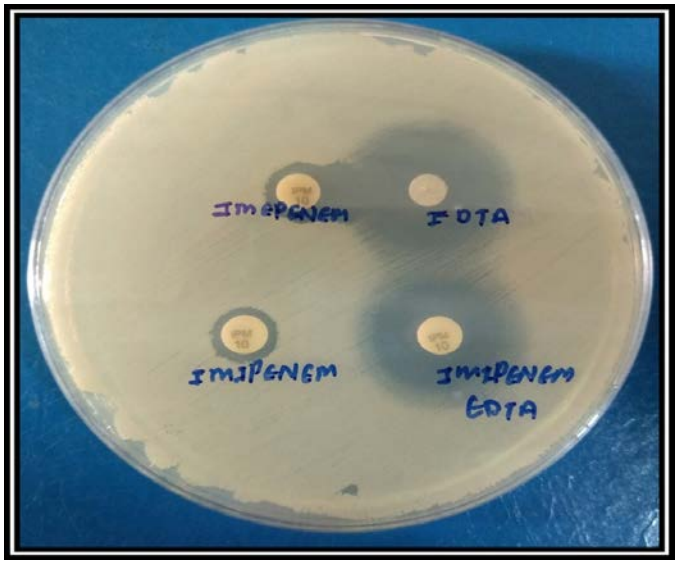

Fig:1- Imipenem-EDTA Combined Disk Test and IMP-EDTA Double Disk Synergy Test.

Screening and Confirmation for MBL Production

All isolates were screened for MBL production by disc diffusion method combined disc test (CDT) using imipenem and EDTA (CDT-IMP) and double disc synergy test (DDST) using imipenem disc and simple

disc with EDTA (DDST-IMP), thirty (30) isolates were screened as MBL positive isolates shown in (Table no. 1). 19 (63.33%) isolates were confirmeds MBL Producing strains Shown in (Table no.- 2).

Table No-1 MBL Positive Screened Isolates

Total GBN	Gbn Screened Positive For Mbl N (%)	Gbn Screened Negative For Mbl N (%)
150	30 (20%)	120 (80%)

Table No -2 MBL Positive Confirmed Isolates

No. Of Imipenem Resistant Strains	MBL Positive Isolates N (%)	MBL Negative Isolates N (%)
30	19 (63.33%)	11 (36.66%)

Table no.3 Comparison of IMP-EDTACombined DiscTest and IMP-EDTA Double Disc Synergy Test.

	Total	Positive	Negative	P value
IMP-EDTA Combined Disc Test	30	19(63.33%)	11(36.66%)	0.017
IMP-EDTA Double Disc Synergy Test	30	09(30%)	21(70%)	

On Comparison between the two test, IMP-EDTA Combined Disk Test was found to be a better test for phenotypic Confirmation of MBL production with a significant P value,Shown in (Table no.- 3)

p value = 0.017 and hence will be consider as significant

Discussion

Antibiotic resistance is a tremendous health problem. This includes the resistance to carbapenemas which was considered as the last resort for Enterobacteriaceae infection[9].However, since last 15 years, acquired resistance to these life saving antimicrobials has been increasingly reported not only in *Pseudomonas aeruginosa* and *Acinetobacter species*, [10]. but also among other member of Enterobacteriaceae. E.coli and Klebsiella pneumoniae are the most common pathogens in Enterobacteriaceae family. These carbapenemas producing bacteria were found in many countries, such as China[11],Pakishtan[21], India[1]),Turkey[1], Brazil[[15], Maxic[16], Peru[17], and Greece[18].This resistance is mainly mediated by MBLs.

In our study *E.coli* 75 (50%) was the most common isolate followed by *Klebsiella species* 31 (21%), *Pseudomonas aeruginosa* 15 (10%), *Citrobacter species* 12 (08%), *Acinetobacter species* 11 (7.33%) and *Proteus species* 6 (04%). However, In another study studies done by Agarwal et al.[19] *Acinetobacter species* (42.10%) was the most common isolate followed by *Pseudomonas aeruginosa* (14.28%), *Proteus* species(10.5%), *Ecoli(* 5.26%0 and *Klebsiella species* (4.76%). Okoche et al,[20] reported Klebseilla species (52.2%), Pseudomonas aeruginosa (31.9%), E. coli (28.4%) to be the most common.

30 Imipenem resistant Gram negative bacterial isolates were tested by the two different screening methods namely combined disc test (CDT) using imipenem and EDTA (CDT-IMP) and double disc synergy test (DDST) using imipenem disc and simple disc with EDTA (DDST-IMP). Of these 19(66.33%) isolates were positive by combined disc method(CDT) using imipenem and EDTA (CDT-IMP) and 9(30%) isolates were positive by double disk synergistic test (DDST) using imipenem disc and simple disc with EDTA(DDST-IMP). Other studies showed similar results of 100% positive result for MBL combined disc method, whereas 93.68% positivity by double disk synergic method. These results are comparable to results of studies done by Stood et al.[21] 100%, Irfan et al. [22] 100%, Attal et al. 88.89%, and Fam et al.

87.5% [23]. The combined disc method using Imipenem + EDTA(CDT-IMP) was found to be superior to Imipenem EDTA double disk synergy test (DDST) p=0.017 which is in concordance with the studies conducted by Yan at al.[6]and Behera at al. [7].

In India most studies have used the imipenem-EDTA combined disc test and double disc synergy test using imipenem-EDTA according to which MBL production ranged from (7-65%)[24]. 68.4% of MBL production was reported by Vinod et al. (Wattal et al, 2010). MBL production has been reported as 70.8% from North India. [25].

Our study showed that Imipenem resistant isolate can be routinely screened for MBL production using simple methods such as Imipenem and Imipenem-EDTA combind disc test which will be crucial step towards large scale monitoring of these emerging resistant organisms. MBL has become a serious problem worldwide and several aspects of them are worrying the community . These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination [26]. They compromise the activity of wide – spectrum antibiotics creating major treatment therapeutic difficulties with significant impact on the outcome of patient appropriate antimicrobial selection, surveillance system and effective infection control procedures are the key factors in their control.

Conclusion

The present study suggests that both tests combined disc test (CDT) using imipenem and EDTA (CDT-IMP) and double disc synergy test (DDST) using imipenem disc and simple disc with EDTA (DDST-IMP) are simple and easy to perform in the laboratory and helpful l in MBL detection in any setup but combined disc synergy test(CDST) is better test as compared to double disc synergy test (DDST) as it is devoid of visual misinterpretation.

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