World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

IDENTIFICATION OF CARBAPENAMASE PRODUCTION IN MULTI DRUG RESISTANT GRAM NEGATIVE BACTERIA BY PHENOTYPIC METHODS FROM A TERTIARY CARE HOSPITAL OF WESTERN U.P. INDIA

[1]*Shivendra Mohan, [2]Umar Farooq and [1]Sana Nudrat

[1]Ph.D. Scholar Department of Microbiology Teerthankar Mahaveer Medical College, Moradabad.
[2]Professor and Head, Department of Microbiology Teerthankar Mahaveer Medical College, Moradabad.

***Corresponding Author: Shivendra Mohan**
Ph.D. Scholar Department of Microbiology Teerthankar Mahaveer Medical College, Moradabad.

Article Received on 23/02/2017 **Article Received on 17/03/2017** **Article Accepted on 07/04/2017**

ABSTRACT

Objectives: To determine the occurrence of carbapenam resistance among MDR-GNB isolates by phenotypic methods. **Introduction:** Cabapenams posses the broadest spectrum of activity against gram positive and gram negative bacteria, and play a critically important role in our antibiotic armamentarium. Detection of carbapenamase enzyme production is a crucial infection control issue because they are often associated with antibiotic resistance, treatment failures and infection associated mortality. **Material and methods:** Study was performed during April 2016 to October 2016. Isolates found resistant to≥3differnt groups of antibiotics were subjected to phenotypic test I.e.; Modified Hodge Test (MHT) and MBL E strip Test for detection of carbapenamase enzyme production. **Results:** Total 105 samples were processed by conventional culture method and antibiotic sensitivity test by Kirby Bauer disk diffusion method and 45 were found resistant to≥3 different groups of antibiotics. Out of 45 resistant cases 09 were positive for MHT and 07 were MBL E Strip test positive. **Conclusion:** Simple, rapid and cost effective test are required to be done on a routine basis for detection of carbapenamase enzymes along with class determination so that appropriate antibiotic treatment can be started in cases of extensive antibiotic resistance.

KEYWORDS: Metallo β lactamase, carbapenamase, multidrug resistance, MDR- GNB.

INTRODUCTION

The relentless threat posed by microbial drug resistance has achieved the dimension of a global pandemic.[1] MDR-GNB can be defined as GNB which are resistant to ≥3 different classes of anti-bacterials.[2,3] Carbapenamases may be defined as beta lactamases that significantly hydrolyze at least imipenem or meropenem.[4] β- lactamases are enzymes produced by certain gram positive and gram negative bacteria that have ability to hydrolyze β lactam drugs. Infections with MDR- GNB are associated with, Mortality rates 21% higher. Results in longer inpatient stays and Higher costs of treatment.[5] PCR amplification and sequencing of β lactamase/ carbapenamse genes (e.g; NDM, VIM, KPC, CTX-M, etc.) can now be used for precise genotypic identification but are expensive and labour intensive and so far restricted to specialized laboratories.[5] Carbapenam hydrolysing β lactams i.e.; carbapenamase, are an increasing concern because firstly, they often also confer resistance to most other β lactams, in addition to their resistance to β lactams MBL producing strains are frequently resistant to aminoglycosides and fluoroquinolones and secondly, unlike carbapenam resistance due to several other mechanisms the resistance due to MBL and other carbapenamase production has a potential for rapid dissemination as it is often plasmid mediated.[7,8] Several recent studies clearly show that resistance to carbapenams is increasing throughout the world.[9] Consequently the rapid detection of carbapenamase is necessary as they are often associated with extensive antibiotic resistance, treatment failures and infection associated mortality. According to centre for disease control infection control guidelines all the carbapenam resistant bacteria should be identified among the isolates so as to initiate effective infection control measures to prevent their dissemination.

MATERIAL AND METHODS

This was a prospective laboratory based study done during April 2016 to October 2016. During this period samples taken from patients admitted in wards and ICU's were subjected to conventional culture method and 105 samples yielded growth of Gram negative Bacteria were studied further. There was no exclusion on basis of type of clinical sample. Out of 105 samples processed 45 were found to be multi drug resistant I.e., resistant to ≥3

different classes of antibiotic by Kirby Bauer disk diffusion method on Muller Hinton agar or showing increased MIC of meropenam by ≥4µg/ml by meropenam E strip test were included for further study.

Tests done for phenotypic detection of carbapenamase production

Modified Hodge Test[10]

Procedure[10]– A lawn culture of overnight suspension of E. coli ATCC 25922 adjusted to 0.5 McFarland Standard was inoculated using sterile cotton swab on to Mueller-Hilton agar. A 10µg imipenem disk was placed at the centre of the plate and the test strain was streaked from edge of the disk to the periphery of the plate in four different directions. The plate was incubated overnight aerobically at 37°C.

Interpretation[10]- The presence of a cloverleaf shaped zone of inhibition by the test strain was considered positive for Carbapenamase production and the negative strain showed an undistorted zone of inhibition. (Fig. 1.1 and Fig.1.2)

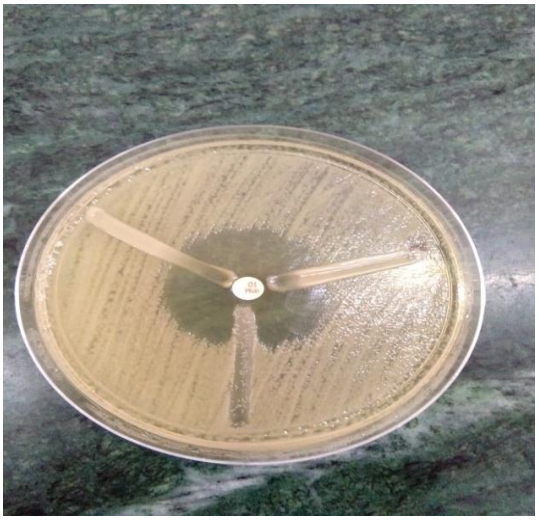

Figure 1.1

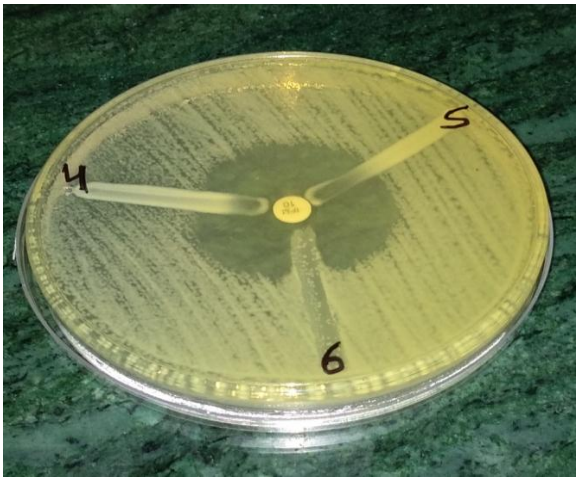

Figure1.2: Strain 6 shows Positive MHT and strains 4, 5 are Negative.

MBL E strip test[11]

Procedure[11]- Prepare plates of Mueller-Hinton agar. Using sterile cotton swab the test strain was inoculated on Mueller-Hinton agar plate. EZY MIC™ strip was placed at the centre of the plate. Within 60 sec. EZY MIC™ strip is adsorbed and will firmly adhere to the agar surface. Plates was incubated overnight aerobically at 37°C.

Interpretation[11]- MBL positive strain - when the ratio obtained for meropenam: value of meropenam + EDTA is more than 8. Or if any size zone is observed on the side coated with meropenam + EDTA and no zone is observed on the opposite side coated with meropenam. In these two conditions strain is considered positive. (fig.2.2) MBL Negative strain- when the ratio of the value obtained for meropenam: value obtained for meropenam + EDTA is less than or equal to 8. (fig2.1)
MBL non conclusive when no zone of inhibition is obtained on either side or if complete inhibition is obtained on both sides then test is considered as non conclusive. (fig2.3)

Figure 2.1: E Test Negative 2.2 E Test Positive 2. 3 E- Test Non Conclusive.

Observation and Result: Out of 105 samples 45 were found resistant to ≥3 different class of antibiotics. So 45 were tested by Modified Hodge test for detection of carbapenamase enzyme detection and MBL E test for detection of Metallo beta lactamase production. Out of 45 samples 9(19.5%) samples showed positive MHT and 7(15.2%) gave positive results for MBL E strip test. Out of 45 MDR –GNB isolates 34 were from enterobacteriaceae and 11 were non fermenters. Carbapenamase producers among enterobacteriaceae family was found 20% (7/34) and among non-fermenters 18.1% (2/11) produced carbapenamase.

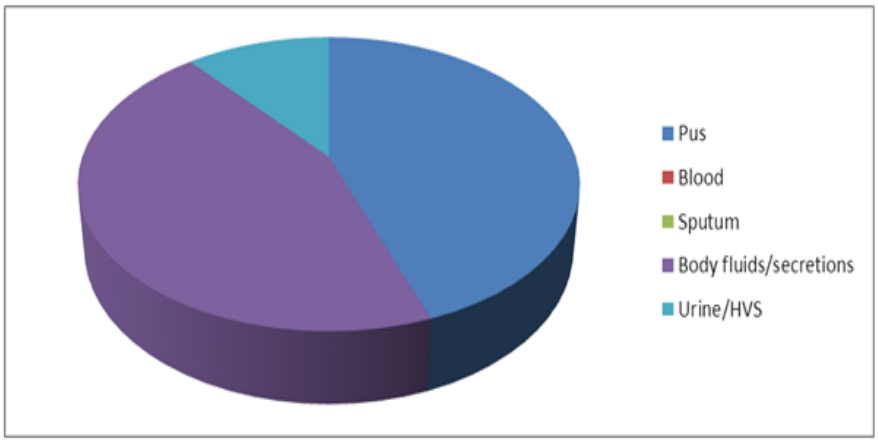

Figure3: Shows sample wise distribution of carbapenamase producing organism.

Table 2: Shows hospital stay of patients with positive and negative carbapenamase production among MDR isolates.(n=45).

Duration in Days	Carbapenamase Positive	Carbapenamase negative
1-10	03	16
11-20	06	14
20-30	00	06
Total	09	36

Patients with >10 days of hospital stay had increased risk of acquiring carbapenamase producingorgainism.

DISSCUSION

Our study shows high occurrence rate of carbapenamase producers which is 19.5% similar findings were reported by Balan K *et al.*[12] who reported 22.5% positivity rate. However, Sahid *et al.*[13] reporeted quite low occurrence rate of carbapenamase producers. In our study 20% carbapenamase producers were from enterobacteriaceae and 18.1% from, acinetobacter species and pseudomonas species which was quite similar to findings reported by Noyal M. J. C. *et al.*[14] who reported 28.1% and 16.2% positive isolates from pseudomonas species and acinetobacter species respectively. Majority of samples in which carbapenamase enzyme was positive were of pus and body fluids and patients who had hospital stay of more then 10 days had increased risk of accquiring carbapenam resistant organism was seen in our study similar findings were reported by Gaikwad Vaishali *et al.*[15] they reported 45.8% cases of MBL producers were from pus.

CONCLUSION

Understanding scope of problem is important for prevention. Detection of carbapenamase enzyme production is a crucial infection control issue because they are often associated with antibiotic resistance, treatment failures and infection associated mortality. In our study high occurrence rate of carbapenamse production is found so Simple, rapid and cost effective test are required to be done on a routine basis for detection of carbapenamase enzymes along with class determination so that appropriate antibiotic treatment can be started in cases of extensive antibiotic resistance. More such studies and clinical trials are required to be done with larger sample size gather a more accurate data on carbapenamase prevalence and its risk factors so that appropriate control measures and hospital infection control policies can be applied.

REFERENCES

1. Shivappa G sowmya et al; Detection of Metallo beta lactamase production in clinical isolates of Nonfermentative Gram negative bacilli. IOSR jour. Of dent. And med. Sci. oct, 2015; 14(10): 43-45.
2. Sameer J. *et al*; A New Metric of Antibiotic class resistance in Gram Negative Bacilli Isolated from Hospitalized children. Jour. Of inf. Control and hosp. epidemiology, June 2012; 33(6): 602-7.
3. Per ljugman *et al*; Transplant infections, 43(4): 677.
4. Ravikant et al; Current concept of New Delhi Metallo beta lactamases (NDM). SMU Med. Jour, July 2014; 1(2): 88-100.

5. Frauke Mattner et al; Dtsch Arztebl Int, 2012; 109(3): 39-45.
6. Yanling xu et al; Epidemiology of carbapenamm resistant enterobacteriaceae (CRE) during 2000-2012 in Asia. J. Thorac. Dis, 2015; 7(3): 376-385.
7. Asthana S *et al;* Detection of carbapenamase production in Gram negative Bacteria. Jour. Of Lab Phy. July – Dec, 2014; 6(2): 69-75.
8. Triphati P *et al*; Prevalence of Multidrug resistance ESBL and MBL production in Acinetobacter species. Int. J. Recent Trends 2013; 6(1): 139-43.
9. Mohammad N *et al*; Phenotypic and Genotypic detection of Metallo β lactamse in imipenam resistant A. *Baumanni* isolated from tertiary care hospital in Alexendria, Egypt. Res. J. Microbiol, 2011; 6(1): 750-60.
10. Modified Hodge test for Carbapenamase Resistant enterobacteriaceae Department of Health and Human Services. CDC Guidelines.
11. Performance standards of Antimicrobial Disc Susceptibility Tests, M100 S21 CLSI, 2011; 31(1).
12. Balan K; Modified Hodge Test and Remodified Hodge Test for carbapenamase detection: Better Indicator Ind. J. App. Res, 2013; 3(3): 279-80.
13. Shahid Md. Et al; Molecular epidemiology of carbapenam resistant enterobacteriaceae from a north Indian tertiary hospital.NZJ Med. Lab Sci, 2012; 66: 5-7.
14. Noyal M.J.C. et al; Simple screening Tests for detection of carbapenamase in clinical isolates of nonfermentative gram negative bacteria. Ind. J. Med. Res, June 2009; 707-12.
15. Gaikwad V et al; Study the prevalence and risk factors of metallo β lactamase producing Pseudomonas aeruginosa from tertiary care centre. Int. J. Sci. res, May 2015; 4(5): 3126-32.