



DETECTION OF NDM,VIM, IMP METALLO- β -LACTAMASE GENES IN KLEBSIELLA PNEUMONIAE ISOLATED FROM CLINICAL SAMPLES AT A TERTIARY CARE CENTRE: A STUDY FROM SOUTHERN RAJASTHAN

Dr. Shubhangi Sharma

Phd Scholar, Department Of Microbiology, R.n.t Medical College, Udaipur-313001, Rajasthan.

Dr. Anshu Sharma

Senior Professor & Guide, Department Of Microbiology, R.n.t Medical College, Udaipur-313001, Rajasthan.

ABSTRACT

Background: The increased prevalence of carbapenem-resistance caused by Metallo- β -lactamase (MBL) is worrisome in clinical & community settings world widely. The mortality rate associated with infections caused by MBLs producing organisms ranging from 18 to 67%. This study aimed to determine the prevalence of Metallo- β -lactamase genes NDM, VIM and IMP among klebsiella pneumoniae isolates in various clinical samples pus, urine, blood samples, respiratory secretions (tracheal & endotracheal), peritoneal-fluid. **Methods:** It is a descriptive cross-sectional study carried out to detect MBL genes NDM, VIM and IMP by combined disc diffusion test and multiplex realtime PCR reactions among 200 klebsiella pneumoniae isolates, at MBGH hospital during Jan.2023 to Jun.2023. The standard microbiological methods were used for the isolation and identification of klebsiella pneumoniae isolates. All the 200 isolates of klebsiella pneumoniae were screened by the carbapenem (Meropenem/Imipenem/Ertapenem)resistant by Kirby Bauer Disc Diffusion method on Muller Hinton Agar. The confirmatory detection of Metallo- β -lactamase (MBL) was done by the combined disc diffusion test (CDT), further all the combined disc test positive isolates were enrolled for RTPCR. **Results:** Out of the 200 klebsiella pneumoniae isolates, 80(40%) were found resistant to carbapenem (Meropenem/Imipenem/Ertapenem). 55 (27.5%) isolates were found positive by combined disc test among all the 80 klebsiella pneumoniae isolates. prevalence of MBL genes by multiplex RTPCR assays among 55 combined disc positive klebsiella pneumoniae isolates, we found 44(22%) isolates positive and 11(5.5%) were found as negative. New Delhi Metallo beta lactamase was the most frequent genes 27(49%), followed by Verona integron Metallo beta-lactamase (VIM) was 11(20%), and consequently, imipenemase (IMP) was 6(10%). There was a statistically, significant association between the combined disc test and the RTPCR assay (Pvalue <1). **Conclusion:** This study revealed the increase in trend of carbapenemase production and a high prevalence of MBL genes in klebsiellae pneumoniae isolates from MBGH Hospital which were not previously established.

KEYWORDS : klebsiella pneumoniae, Metallo- β -lactamase (NDM,VIM, IMP), CDT, RTPCR

INTRODUCTION:

Carbapenem are β -lactams group of antimicrobial agent, most oftenly used as the last resort antibiotics to treat life-threatening infections caused by multidrug resistant microbes.^{1,2} However, in the recent past,due to increasing indiscriminate use of carbapenems, resistance have been reported ,which are quite alarming.³Carbapenemases production & reduced expression of porins along with the overproduction of β -lactamases possessing weak carbapenemase activity, these are two mechanisms responsible for carbapenem resistance.⁴ β -lactamases are divided into 3 classes which are Ambler classes A, B and D β -lactamases. Class B β -lactamases require one or two zinc ions for their full catalytic activity and these enzymes are called metallo-beta-lactamase (MBL).⁵The most common Class B metallo- β -lactamase families include the New Delhi metallo- β -lactamase (NDM), imipenem-resistant Pseudomonas (IMP) verona integron-encoded metallo- β -lactamase (VIM),German imipenemase, and Seoul imipenemase.⁶

Objectives:

The aim of the study was to investigate the presence of Metallo- β -lactamase (MBL) carbapenemases in klebsiella pneumoniae in our hospital settings and to evaluate the efficiency of phenotype and molecular assay methods for the detection of MBL production.To determine the prevalence of classB carbapenemases (Metallo- β -lactamase) by phenotypic confirmatory combined disc diffusion test. To determine the existence of blaNDM,blaVIM,blaIMP gene in all combined disc positive k.pneumoniae isolates by RTPCR molecular assay.

MATERIALS AND METHODS:

It's a descriptive cross-sectional study, carried out to detect Metallo- β -lactamase (MBL) blaNDM, blaVIM and blaIMP by combined disc test and multiplex RTPCR reactions among 200

klebsiella pneumoniae isolates, at MBGH hospital during Jan.2023 to June 2023.The standard microbiological methods were used for the isolation and identification of klebsiella pneumoniae isolates. All the 200 isolates of klebsiella pneumoniae were screened by the carbapenem resistance by Kirby bauer disc diffusion method on Muller Hinton Agar.

All the isolates which were found to be carbapenem-resistant by screening test were subjected to phenotypic confirmatory test in the form of Combined disc test (CDT),further all the combined disc test positive Metallo- β -lactamase (MBL) blaNDM, blaVIM and blaIMP isolates were enrolled for polymerase chain reaction.

RESULTS:

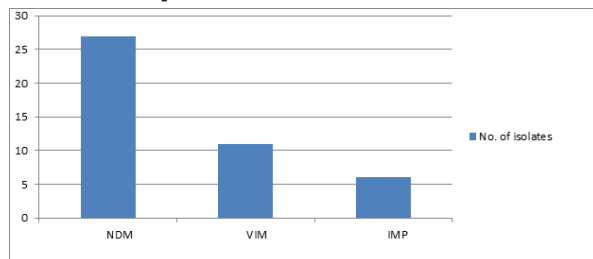
Out of the 200 klebsiella pneumoniae isolates,80(40%) were found resistant to carbapenem by antibiotic susceptibility testing. Among 80 carbapenem resistant klebsiella pneumoniae isolates we were found 55 (27.5%) isolates positive for MBL (classB) & 04(2%) isolates were found positive for KPC(classA) by Combined disc test

Carbapenem ase gene	PBA	EDTA	No. of positive isolates
KPC	+	-	04
MBL	-	+	55
KPC+MBL	-	-	21

Out of 200 klebsiella pneumoniae isolates,55 MBL positive isolates by Combined disc test could be preserved in pure form, which were used for carbapenemase gene(NDM,VIM, IMP) detection by RTPCR.

22%(44) isolates were positive by RTPCR & rest 11 were found negative for any other (NDM,VIM,IMP) gene by RTPCR.New Delhi Metallo beta lactamase was the most frequent genes 27(13.5%),followed by Verona integron Metallo beta-lactamase (VIM) was 11(5.5%), and consequently, presence

of imipenemase was 6(3%). There was a statistically significant association between the combined disc test and the RTPCR assay (P value <1)



DISCUSSION:

The emergence of multidrug bacterial resistance due to carbapenemase production is increasing day by day worldwide.⁷ This is more crucial to rapid identification of carbapenem resistance, especially in Indian set up because predominance of blaNDM-1 as carbapenemase encoded gene in the multidrug resistant isolates.⁸ In the present study, 80(40%) isolates of *Klebsiella pneumoniae* were screened to be positive for carbapenem resistance. Satyajit K Pawar et al. (31.77%)⁹, Kumarasamy et al. (24%)⁹ and Panchal et al. (28%)¹⁰ also reported a higher carbapenem resistance rate.

We found MBL production in 55 (27.5%) isolates of *Klebsiella* by using CDT, a similar study done by Panchalet al. (19.62%)¹¹ and Adekar et al. (18.00%)¹². In contrast to our study, Fazlul et al. (52.6%)¹² and Marie et al. (53%)¹³ have found a high prevalence of MBL in their studies.

In this study we found 44(22%) isolates were positive for NDM, VIM, IMP genes by RTPCR. New Delhi Metallo beta lactamase was the most frequent genes 27(13.5%), followed by Verona integron Metallo beta-lactamase (VIM) was 11(5.5%), and consequently, imipenemase (IMP) was 6(3%). Various authors from India have reported a high prevalence of NDM in their studies. Gupta et al.¹³ reported a 17%–22%, Vaz¹⁴ reported 26%, Pawar et al.¹⁵ from Karda reported a prevalence of 31.77

SUMMARY AND CONCLUSION:

This study revealed the increase in trend of carbapenemase production and a high prevalence of MBL genes in *Klebsiella pneumoniae* isolates, which were not previously established. There is an urgent need to implement multidisciplinary approach to limit the spread strategies for early detection, antimicrobial stewardship programme & appropriate treatment measures are required to stop the spread of carbapenem-resistant organisms are the need of the hour to successfully tackle the global threat.

REFERENCES:

- Shobha KL, Lenka PR, Sharma MK, Ramachandra L, Bairy I. Metallo- β -lactamase production among *Pseudomonas* species and *Acinetobacter* species in coastal Karnataka. *J Clin Diagn Res* 2009;3: 1747-53.
- Sekar R, Mythreyee M. Carbapenem resistant enterobacteriaceae: A growing threat to public health. *Open Access Sci Rep* 2012;1:324. [doi: 10.4172/scientificreports.324]
- Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! *Trends Mol Med* 2012;18:263-7
- Pitout JD, Laupland KB. Extended-spectrum β -lactamase-producing Enterobacteriaceae: An emerging public-health concern. *Lancet Infect Dis* 2008;8:159-66.
- Schlesinger SR, Lahousse MJ, Foster TO, Kim SK. Metallo- β -lactamases and aptamer-based inhibition. *Pharmaceuticals (Basel)* 2011;4:419-28.
- Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010;10:597-602.
- Fazlul MKK, Deepthi S, Farzana Y, Najnin A, Rashid MA, Munira B, et al. Detection of metallo-lactamases-encoding genes among clinical isolates of *Escherichia coli* in a tertiary care hospital, Malaysia. *Int J Pharmaceutical Res* 2019;11:2908.
- Chatterjee B, Khanduri N, Kakati B, Kotwal A. Universal Presence of blaNDM-1 Gene in carbapenem-resistant gram-negative bacilli in an Indian hospital in

2015. *J Clin Diagn Res*. 2017;11(9):DL01-DL02
- Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010;10:597-602
- Panchal CA, Oza SS, Mehta SJ. Comparison of four phenotypic methods for detection of metallo- β -lactamase-producing gram-negative bacteria in rural teaching hospital. *J Lab Physicians* 2017;9:81-3.
- Wadekar MD, Anuradha K, Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. *Int J Current Res Acad Review* 2013;1:89-95
- Marie MA, John J, Krishnappa LG, Gopalakrishnan S. Molecular characterization of the β -lactamases in *Escherichia coli* and *Klebsiella pneumoniae* from a tertiary care hospital in Riyadh, Saudi Arabia. *Microbiol Immunol* 2013;57:805-10.
- Gupta V, Garg R, Kumaraswamy K, Datta P, Mohi GK, Chander J. Phenotypic and genotypic characterization of carbapenem resistance mechanisms in *Klebsiella pneumoniae* from blood culture specimens: A study from North India. *J Lab Physicians* 2018;10:125-9.
- Nair PK, Vaz MS. Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai. *Indian J of Microbiology and Infectious Diseases*. 2013;3(4):207-210.
- Pawar SK, Mohite ST, Shinde RV, Patil SR, Karande GS. Carbapenem-resistant Enterobacteriaceae: Prevalence and bacteriological profile in a tertiary teaching hospital from rural western India. *Indian J Microbiol Res*. 2018;5(3):342-347.