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Study of New Delhi Metallo Beta Lactamase and Oxacillinase beta Lactamase coexistence in Carbapenem Resistant Klebsiella Pneumoniae Obtained from Bloodstream Infections

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ABSTRACT

Background: Carbapenem-resistant Klebsiella pneumoniae (CRkp) is of utmost clinical importance because they challenge the armamentarium of the treating clinicians, hampering current treatment strategies. This study aimed to compare the outcomes of oxacillinases β-lactamases (OXA-48) and New Delhi metallo-β-lactamase (NDM-1)-producing CRKP isolates, the two most common carbapenemases reported from Worldwide including India, obtained from patients with bloodstream infections in an ICU in a tertiary care center in southern part of Rajasthan and to compare the different laboratory methods for their detection.

Materials and methods: Klebsiella pneumoniae isolates obtained from the blood culture of patients admitted to various ICUs were subjected to screened for carbapenem resistant detection by Combined Disc Test and subsequently by real time multiplex polymerase chain reactions. Antibiotic susceptibility tests (AST) were performed and clinical data were recorded.

Results: A total of 91 Klebsiella pneumoniae isolates were obtained from the blood culture. 63 isolates were showing resistance for carbapenem(meropenem/imipenem/ertapenem) by Antibiotic susceptibility test on Mueller Hinton Agar. In Combined Disc test 40 isolates were found positive for class B group of carbapenemases. Among 63 carbapenem resistance isolates, 50(79%) were found positive rest 13(20%) reported as negative by real time Multiplex RTpcr. There were 23(36%) isolates showing with NDM and OXA β-Lactamases coexistence in Carbapenem-Resistant Klebsiella Pneumoniae. Statistically significant differences were found in the AST pattern between the isolates with two genes.

Conclusion: This study revealed the increase in trend of carbapenemase production and a high prevalence of coexistence of different carbapenemase gene that's why Surveillance of carbapenemase genes in a hospital setting is essential.

Keywords: Carbapenem-resistant Klebsiella pneumoniae (CRkp), oxacillinase β-lactamases (OXA-48) and New Delhi metallo-β-lactamase (NDM), Combined Disc test, real time Multiplex RTPCR

INTRODUCTION

In India, the rates of carbapenem resistance have seen a sharp increase of 35% in just two years (9% in 2008 to 44% in 2010) [1]. Likewise, the rates in Italy soared to 60% in 2013 from undetectable levels in 2008. [2] Approximately 8% of all hospital associated infection & 14% of cases of primary

bacteremia reported annually, in k. pneumoniae strains the emergence & dissemination of drug resistance have made the situation even more worse. In India 80% of all k. pneumoniae strains are resistant to cephalosporin & up to 60% are carbapenem resistant has been observed. Carbapenem resistance is primarily mediated by the production of carbapenemases enzyme, which have been found in *K. pneumoniae* isolates to fall into three categories: (1) metallo-ß-lactamases or molecular class B lactamases (New Delhi metallo-ß-lactamase, IMP, VIM etc.) that hydrolyze all lactams except monobactams and are inhibited by ethylenediaminetetraacetic acid (EDTA) but not clavulanic acid; (2) serine –ß-lactamases of molecular class A(SME, KPC, and GES etc.) that hydrolyze even monobactams are inhibited by clavulanic acid and tazobactam but not by EDTA; (3) molecular class D serine- β-lactamases (oxacillinases β-lactamases {OXA-48}) that do not hydrolyze monobactams and are poorly inhibited by clavulanic acid and EDTA[3]. These properties are used for differentiation in the laboratory. Most of these carbapenemases are acquired either by mutation or by horizontal gene transfer. Carbapenemase-producing Klebsiella pneumoniae (CRKP) has become a menace in several intensive care units, which needs to be controlled immediately after being reported by a laboratory. Due to the limited therapeutic options available and mortality rates of as high as 40-50% have been reported.[3]

Among all of these classes of carbapenemases, New Delhi Metallo-β-Lactamase and Oxacillinases β- Lactamases have been reported to be the commonest in India. Phenotypic tests are presently being used in many bacteriology laboratories to differentiate between these genes, but the efficiency of these tests in routine laboratory settings are sparsely known. Therefore, the present study aimed to evaluate the efficiency of phenotypic (combined disc test) and multiplex RTPCR molecular assay methods for the detection of MBL production as well as to find the prevalence of NDM & OXA-48 β-Lactamases gene coexistence in carbapenem-resistant klebsiella pneumoniae from bloodstream infections in an ICU at tertiary care center in Southern Rajasthan.

MATERIAL & METHOD

Study design and settings:

It's a cross sectional descriptive study carried out in the Department of Microbiology, R.N.T Medical College Hospital, Udaipur during a period of May 2023 to December 2023 on ninety one Klebsiella Pneumoniae isolates obtained from the blood culture of patients admitted to ICU of MBGH were subjected to phenotypic (combined disc test) as well as molecular assay (multiplex RTPCR) .The standard microbiological methods were used for the isolation and identification of klebsiella pneumoniae isolates[4]. All the 91 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[5] (CDT) & Multiplex RT PCRs were done to detect blaNDM and blaOXA48-like genes. The combined disc test was performed as described by Pournaras S et al[17] with use of meropenem(10µg) disc[17]. The test organisms were inoculated on Muller Hinton agar plates as per CLSI guidelines. After 10 minutes, using four 10-µg meropenem (MER) disks (Himedia), including a MER disk alone, a MER disk plus 10 µl of 40 mg/ml phenyl boronic acid (PBA) (Himedia) for KPC inhibition, a MER disk plus 10 µl of 0.1 M EDTA (Himedia) for MBL inhibition, and a MER disk plus both PBA and EDTA for simultaneous inhibition of KPC (by PBA) and MBL (by EDTA). MER disks were set about 30 mm apart. The inhibition zone of ≤25 mm around the MER disk alone was the optimal breakpoint, exhibiting the best

performance for carbapenemase producing Klebsiella pneumoniae detection. A difference of ≥5 mm in the inhibition zone between the MER disk without and with the inhibitors (PBA, EDTA, or both), taking also into account scattered colonies within the inhibition halo, was considered a positive result for the detection of KPC, MBL, or both carbapenemases, respectively[16].

Molecular Detection of *bla*NDM, *bla*OXA-48, *bla*KPC by Multiplex PCR

Confirmation of Carbapenem resistant isolates was done by gold standard method that is multiplex PCR. Plasmid DNA was extracted from the screened positive 63 isolates by using the HiPurA® Bacterial Genomic DNA Purification Kit as per the manufacturer's instructions. Plasmid DNA concentrations were determined. The PCR was first optimised to obtain all possible amplicons according to described protocols (HiPurA® Bacterial Genomic DNA Purification Kit). The extracted plasmid DNA of each isolate were subjected to multiplex PCR of the *bla*NDM, *bla*OXA-48, *bla*KPC genes by using the Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit. For multiplex PCR, 25 µL PCR reaction mixture included 12.5 µL of master mixture CRG1 Tube & 12.5 µL of master mix CRG2 Tube,4 µL µL of each primer in each tube, Molecular Biology Grade Water for PCR 1.5 µl in each tube, 1 µL of Internal Control Primer-Probe Mix, Internal Control DNA in both CRG1 Tube CRG2 tube.5µL volume of Positive Control / Negative Control /Template DNA in the both tubes. The PCR amplification programme was performed as per the following sequence; total of 45 cycles of multiplex PCR consisting initial denaturation step for 10 minutes at 95°C, denaturation step for 05 seconds at 95°C, annealing and extension step for 60°C for 1 minute(plate read) & hold at 4°C for ∞ with a BioRad CFX96 PCR analyzer.

RESULTS:

Ninety one Klebsiella pneumoniae isolates from blood culture specimen were included in this study Among the 91 isolates, sixty three(69.23%) isolates were showing resistance for carbapenem (meropenem/imipenem/ertapenem) by antibiotic susceptibility test on Mueller Hinton Agar by Kirby Bauer Disc Diffusion Test. Forty (43.95%) isolates were found positive for class B group of carbapenemases out of 91 k. pneumoniae isolates by Combined Disc test and none of isolate was positive for *Klebsiella pneumoniae* carbapenemase (KPC) alone & both Klebsiella pneumonia carbapenemase (KPC) & Metallo- β-lactamases (MBL) combinations.

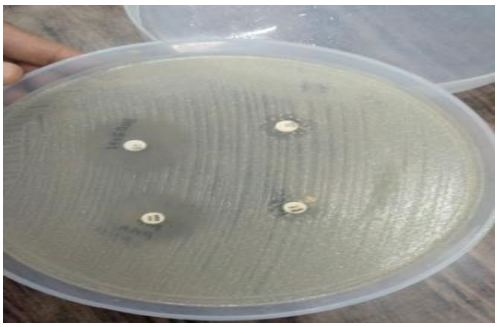


Figure1. Combined disc test showing the zone of enhancement around the M+EDTA Disc (considered as MBL positive)

Tab: 1 Distribution of β-lactamases by combined disc test[6] (phenotypic method):

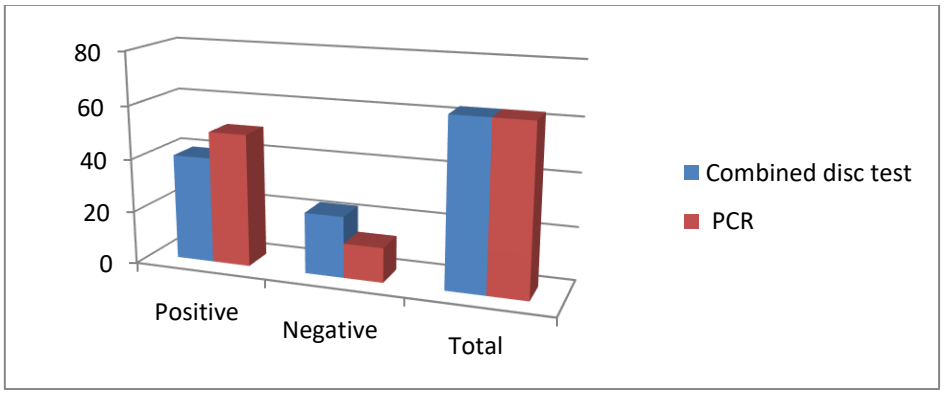
ß-lactamase	PBA	EDTA	No. of isolates(n)
KPC	+	_	00
MBL	_	+	40
KPC+MBL	+	+	00
	_	_	23
Total			63

KPC = Klebsiella pneumoniae carbapenemase, MBL = Metallo β-lactamases, PBA = phenyl boronic acid, EDTA = Ethylene diamine tetra acetic acid

Among sixty-three carbapenem resistance isolates, fifty (79.36%) were found positive by Multiplex RTPCR while as remaining thirteen (20%) isolates reported as negative. In the present study, the phenotypic combined disc test was found to have only moderate sensitivity (77.6%) and negative predictive value (78.9%) as compared to the multiplex RTPCR. Hence considering PCR as the gold standard, the sensitivity and specificity of phenotypic combined disc test were found to be 77.6% (95% CI = 63.4-88.2%) and 100% (95% CI = 91.4-100%) respectively. Positive and negative predictive values were 100% and78.9%, respectively, and the overall accuracy was calculated as 87.8% (95% CI = 79.2-93.7%).

Tab: 2 Comparison between multiplex RTPCR & Combined Disc Test

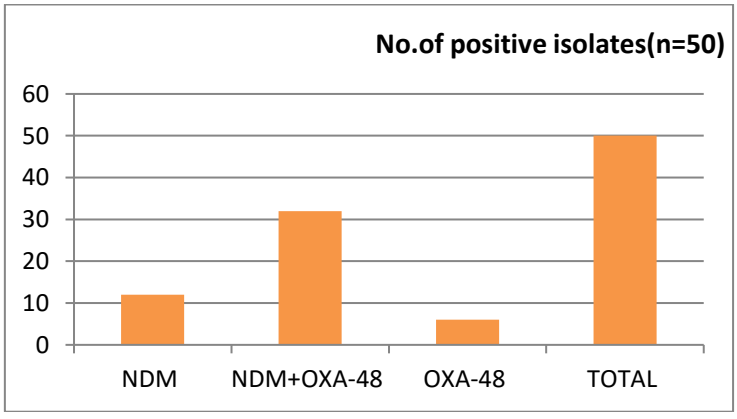
	Combined disc test	multiplex RTPCR
Positive	40	50
Negative	23	13
Total	63	63



Tab: 3 Distribution of Carbapenemase gene detected by multiplex Real-Time PCR

Carbapenemase gene	No. of positive isolates(n)
NDM	12
NDM+OXA-48	32
OXA-48	06
TOTAL	50

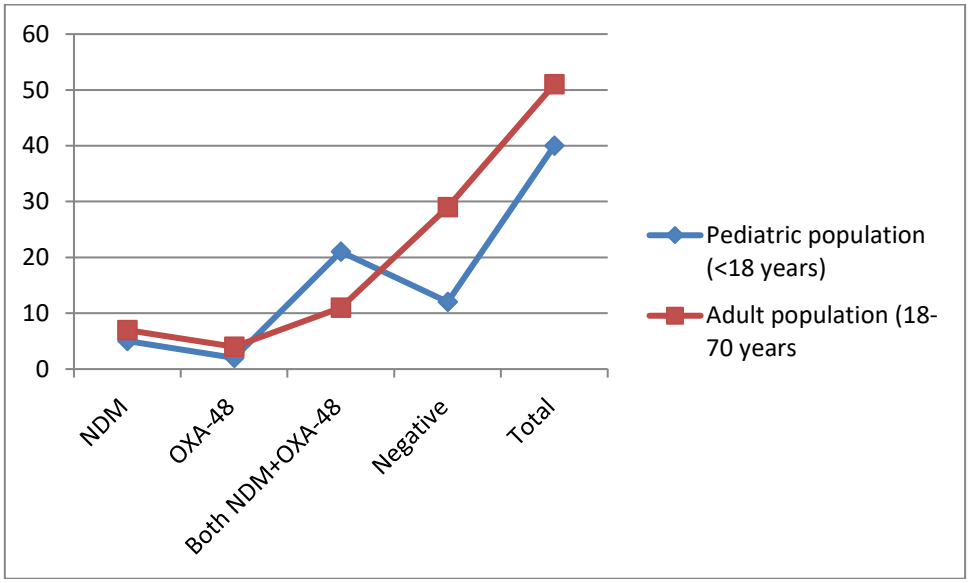
NDM and OXA-48 genes coexistence were detected in 32 (64%) isolates followed by 12(24%) & 06(12%) New Delhi Metallo beta lactamase and oxacillinases gene(oxa-48) was found respectively without coexistence of any other gene among all the 50 PCR positive cases.



Tab: 4 Distribution of carbapenemase gene in different age groups:

ß-lactamase gene	Pediatric population (<18years)	Adult population (18-70 years)
NDM	5	7
OXA-48	2	4
Both NDM+OXA-48	21	11
Negative	12	29
Total	40	51

Ninety-one specimens included in the study, contains forty (43.95%) samples belongs to pediatric population(<18yr.) and fifty one (56%) samples belongs to adult population (18-70yr.). coexistence of New Delhi Metallo beta lactamase and oxacillinases gene(oxa-48) were most common finding in both of the populations. Twenty one (23%) samples were found positive in paediatric population while as eleven (12%) samples were found positive for coexistence of New Delhi Metallo beta lactamase and oxacillinases genes among the 91 of total suspected cases of bacteremia followed by New Delhi Metallo beta lactamase gene five (5.49%) in paediatric population & seven (7.6%) in adult population. Oxacillinase gene found without coexistence in two (2%) paediatric cases and four (4%) in adult cases. twenty-nine (31.86%) samples of adult & twelve (13%) samples of <18yr. age group population were found negative for none of these genes.



DISCUSSION

Emergence of infection associated with carbapenem-resistant K. pneumoniae is a dreaded complication associated with several risk factors. It's a matter of serious concern in developing countries due to significant potential impacts on antibiotic usage and patient outcomes[7]. In this study out of 63 carbapenem resistant isolates by kirby bauer disc diffusion method, 40 (63.5%) showed positive for MBL production but none of the isolates showed KPC production phenotypically by combined disc test, similar finding has been highlighted in a recent study by Mohan B et al from North India regarding absence of KPC enzyme[8]. We were not able to show any Class D enzyme also as till now there is no phenotypic method for Class D enzyme Detection. However, recently a disc diffusion test Temocillin has been shown to be a good indicator of OXA-48, but this needs further evaluation.[9],[10] The present study showed that coexistence of NDM & Oxacillinase-48 ß-lactamase gene are the most common carbapenemases in our

hospital setting. Corroborating the present study, various part of India detected co-production of NDM with Oxacillinases[7],[11] We found NDM[12]gene followed by oxacillinase[13]gene most prevalent, which are similar to study conducted by Veeraghavan et al[1]. Among all the fifty positive isolates,(by molecular assay) NDM gene singly or in combination with Oxa-48 were found more prevalent, and showed absence of any other MBL gene, so this seems to be the most prevalent MBL type of enzyme in our setup, NDM has been found not only in hospital but community settings also[14],[15].In our study, we found that isolates with only blaNDM gene were significantly more susceptible to aminoglycosides and ciprofloxacin and those with blaOXA48-like gene only were significantly more susceptible to trimethoprim-sulphamethoxazole, this is concordant finding to a previous study done by Verma et al in Aug.2019[12]

CONCLUSION

Carbapenems are one of the most commonly used important last resort antibiotics in the treatment of severe infections caused by multidrug-resistant microorganism. Now a days increase in trend of carbapenemase production and a high prevalence of coexistence of different carbapenemase gene It is essential to maintain the clinical efficacy of carbapenems by early detection of carbapenemases and that's why Surveillance of carbapenemase genes in a hospital setting is essential.

Declaration by Authors

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Conflict of Interest: None

REFERENCES

1. Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R: Carbapenem resistant Klebsiella pneumoniae isolated from bloodstream infection: Indian experience. Pathog Glob Health. 2017,111:240-6.10.1080/20477724.2017. 1340128.Epub2017 jul 2.
2. Performance standards for antimicrobial susceptibility testing: informational supplement (2018). Accessed: April 30, 2022: https://mmrc/CLSI-2018-M100-S28.pdf.
3. van Duin D, Kaye KS, Neuner EA, Bonomo RA: Carbapenem-resistant Enterobacteriaceae: a review of treatment and outcomes. Diagn Microbiol Infect Dis. 2013, 75:115-20. 10.1016/j.diagmicrobio.2012.11.009
4. Nordmann P: Carbapenemase-producing Enterobacteriaceae: overview of a major public health challenge. Med Mal Infect. 2014,44:51-6. 10.1016/j.medmal.2013.11. 007.Epub2013Dec.18
5. Giske CG, Gezelius L, Samuelsen, Warner M, Sundsfjord A, Woodford N. A sensitive and specific phenotypic assay for detection of metallo-s-lactamases and KPC in *Klebsiella pneumoniae with the use of meropenem disks supplemented with* aminophenylboronic acid, dipicolinic acid and cloxacillin. ClinMicrobiol Infect 2011;17:552-6.
6. Gupta V, Kumarasamy K, Gulati N, Garg R, Krishnan P,Chander J. AmpC s-lactamases in nosocomial isolates of *Klebsiella pneumoniae from India. Indian J Med Res 2012; 136:237-41.*
7. Kumarasamy K, Kalyanasundaram A: Emergence of Klebsiella pneumoniae isolate co-producing NDM-1with KPC-2 from India. J Antimicrob Chemother. 2012,67:243 4.10.1093/jac/dkr431.Epub 2011 Oct11.
8. Mohan B, Hallur V, Singh G, SandhHK, Appannanavar SB,Taneja N. Occurrence of blaNDM-1 and absence of blaKPCgenes encoding carbapenem resistance in uropathogens froma tertiary care centre from North India. Indian J Med Res2015; 142:336-43
9. Maurer FP, Castelberg C, Quiblier C, Bloemberg GV, Hombach M. Evaluation of carbapenemase screening and confirmation tests with *Enterobacteriaceae and development of a practical diagnostic* algorithm. J Clin Microbiol 2015; 53:95-104.
10. van Dijk K, Voets GM, Scharringa J, Voskuil S, Fluit AC,Rottier WC, *et al. A disc diffusion assay for detection of class A,* Band OXA-48 carbapenemases in *Enterobacteriaceae using phenyl* boronic

acid, dipicolinic acid and temocillin. Clin Microbiol Infect2014; 20:345-9.
11. Doi Y, O'Hara JA, Lando JF, Querry AM, Townsend BM, Pasculle AW, Muto CA: Co-production of NDM-1and OXA-232 by Klebsiella pneumoniae. Emerg Infect Dis. 2014, 20:163-5. 10.3201/eid2001.130904
12. Verma A, Jain P, Tripathi P, et al. (July 24, 2022) Outcomes in Oxacillinases β-Lactamases (OXA-48) and New Delhi Metallo-β-Lactamase (NDM-1)-Producing, Carbapenem-Resistant Klebsiella Pneumoniae Isolates Obtained from Bloodstream Infections. Cureus 14(7): e27197. DOI10.7759/cureus.27197
13. Giani T, Pini B, Arena F, et al.: Epidemic diffusion of KPC carbapenemase-producing Klebsiella pneumoniae in Italy: results of the first countrywide survey, 15 May to 30 June 2011. EuroSurveill. 2013, May30;18(22):20489.
14. Batra P, Dwivedi M, Sherwal BL, Dutta R, Gupta S. NDM-1 Infection and colonisation in critically ill patients from Delhi: A glimpse of the community scenario. Indian J Med Microbiol. 2016; 34:120–1. doi: 10.4103/0255-0857.167679. [DOI] [PubMed]
15. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP: Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol.2008 Dec;29(12): 1099-106.doi:10.1086/592412.
16. Tsakris A, Poulou A, Pournaras S, Voulgari E, Vrioni G, Themeli-Digalaki K, Petropoulou D, Sofianou D. 2010. A simple phenotypic method for the differentiation of metallo-beta-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. J. Antimicrob. Chemother. 65:1664–1671
17. Pournaras S, Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A,Petropoulou D, Sofianou D. 2009. Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumonia* isolates in the clinical laboratory. J Clin Microbiol 47:362–367. http://dx.doi.org/10.1128/JCM.01922-08.

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