Research article

Molecular characterization of carbapenemase production in clinical isolates of *Klebsiella* species isolated in a tertiary care hospital

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ABSTRACT

Introduction and Aim: Emergence of resistance to carbapenems in clinical isolates of *Klebsiella* species is a matter of concern. This prospective study was carried out to determine the prevalence of carbapenem resistance in clinical isolates of *Klebsiella* species phenotypically and to confirm for the presence of bla NDM (New Delhi metallo-β-lactamase) and bla KPC (*Klebsiella pneumoniae* carbapenemase) genes in these isolates.

Materials and Methods: The 336 clinical isolates of *Klebsiella* species were tested for carbapenemase production by phenotypic tests; the Modified Hodge Test (MHT) and Combined Disc Test (CDT). The carbapenemase producers were further confirmed for presence of bla NDM and bla KPC genes using polymerase chain reaction (PCR).

Results: Resistance to carbapenem was seen in 34.52% of clinical isolates of *Klebsiella* species and majority of these isolates were from inpatient units (59.23%) of the Hospital. Maximum cases were seen in males (63.69%) and the positivity rate was high in > 61 years of age (27.08%). On genotypic characterization, bla NDM was the predominant (15.52%) gene detected as compared to bla KPC (10.34%) gene. However, these 2 genes could not be detected in some isolates suggesting other genes responsible for carbapenem resistance.

Conclusion: Emergence of resistance to carbapenem is a matter of concern. There was predominance of bla NDM gene in our hospital. Timely and accurate detection of resistance and rational use of second and third-line antibiotics would allow the early initiation of treatment for better patient outcome.

Keywords: Antibiotic resistance; carbapenemase enzyme; bla KPC gene; bla NDM gene; *Klebsiella* species.

INTRODUCTION

Multi drug resistant (MDR) *Klebsiella* species is becoming more and more difficult to treat because of their ability to spread rapidly, thus tend to cause infections in the hospital settings (1-3). The transmission of *Klebsiella* is due to two main sources that are the gastrointestinal tract and the hands of healthcare workers (1). The known mechanisms of resistance in *Klebsiella* species are extended spectrum beta lactamases, cephalosporinases and carbapenemases production (4). Carbapenems are considered as the high-end antibiotics available in the treatment of critical cases of infection caused by this organism (5). The enzyme carbapenemases are divided into various classes like class A carbapenemases (*K. pneumoniae* carbapenemase KPC types), class B or metallo-beta-lactamases (MBLs) (VIM, IPM, and NDM types), and class D oxacillinases (e.g., OXA-48-like enzymes) (5).

New Delhi metallo-β-lactamase (NDM) and KPC gene are two most common genes responsible for production of resistance to carbapenems. Besides these enzymes, another mechanism responsible for carbapenem resistance has been studied in Enterobacteriaceae (6). Thus, leading to reduction in the number of choices of antibiotics and increasing the chances of treatment failure (5).

This study was carried out to determine the molecular characterization of carbapenemase production in clinical isolates of *Klebsiella* species isolated in a tertiary care Hospital so that effective measures can be taken for better patient outcome.

MATERIALS AND METHODS

A total of 336 clinical isolates of *Klebsiella* species obtained from different clinical samples (pus, blood, sputum, urine, *etc.*) received in microbiology laboratory from various inpatient units and outpatient departments for a period of one year was identified by standard bacteriological methods (7). Susceptibility to various antibiotics was tested as per CLSI recommendations (8).

Detection of carbapenemases

Screening for detection of carbapenemases was carried out by Ertapenem (ETP) using the Vitek 2 Compact system (bioMerieux, France). The screen positive isolates with MIC ≥18 µg/ml for ETP (8) were subjected for confirmation of carbapenemases by two different phenotypic tests i) Modified Hodge Test (MHT) and ii) Combined Disc Test (CDT). The *K. pneumoniae* ATCC BAA-1705 (MHT positive) and *K. pneumoniae* ATCC BAA-1706 (MHT negative) were used for quality control.

Modified Hodge test (MHT)

MHT was as per the recommendation of CLSI (7). The isolate showing a clover leaf-like indentation of *E. coli* 25922 growing along the test organism growth streak within the disk diffusion zone was considered MHT positive and isolate showing no growth of *E. coli* 25922 was taken as MHT negative.

Combined disc test using Imipenem (IPM) and IPM – EDTA disk

The CDT was performed as per the method by Yong *et al*. (10), using IMP disk alone and IPM-EDTA disk. A zone diameter of IPM – EDTA ≥ 7 mm was considered as an MBL producer. The carbapenemase producing isolates were also tested for resistance to colistin.

Detection of blaNDM and blaKPC genes

Further, the MHT and CDT positive isolates of *Klebsiella* spp. Were subjected for detection of blaNDM and blaKPC genes. The bacterial DNA was isolated and then amplified using the gene primers and then gel electrophoresis was done to detect the presence of genes in the DNA. Briefly, amplification of the NDM gene was performed using the forward and reverse primer pairs. Forward primer NDM-1gf 5′-ACC GCC TGG ACC GAT GAC CA-3′ (positions 80–99) and reverse primer NDM-1gr 5′-GCC AAA GTT GGG CGC GGT TG-3′ (positions 343–324) was used which gave rise to an amplicon size of 264 bp. Similarly, amplification of the KPC gene was performed using the forward and reverse primer pairs. Forward primer: 5' TCG CTA AAC TCG AAC AGG 3' and (reverse primer): 5' TTA CTG CCC GTT GAC GCC CAA TCC 3' which gave rise to an amplicon size of 782bp.

For the PCR; the first step was denaturation for 5 min at 94°C, 35 cycles performed with denaturation, annealing and extension for 30 s at 94°C, 30 s at 60°C and for 1 min at 72°C respectively. This was followed by a final extension at 72°C for 10 minutes.
The products of PCR were analysed by gel electrophoresis on 1.5% agarose gel followed by ethidium bromide staining and was viewed under UV light (11-13) to detect the specific amplified products of 264 bp and 782 bp. A 100-bp DNA ladder was used as a standard molecular weight marker. Once the electrophoresis was completed the gel was observed in a UV machine connected to the computer. The photographs of the gel showing the presence of genes were taken and saved for further reference (Fig. 1 & 2, 3 & 4).

Statistical analysis

Data was analyzed in the software Systat version 13.2. Chi-square test was used to see the distribution of carbapenem screen positive *Klebsiella* species in genotypic tests for NDM gene and KPC gene, at 5% level of significance.

Ethical approval

Approval from the Institutional Ethics Committee was obtained via Letter No. SMC/IFC/2019/90/01, dated: 04.03.2019).

RESULTS

The *Klebsiella* spp. Was isolated more frequently from samples received from inpatient units (81.54%). Among the samples, urine was the predominant (30.95%) followed by pus (19.64%), sputum (17.26%) and blood (14.29%) (Table 1). The *Klebsiella* spp. Was isolated more 91 (27.08%) from elderly patients above 61 years of age and in males (63.69%) with a male: female ratio of 1.75:1.

A total of 116/336 (34.52%) isolates of *Klebsiella* spp were resistant to carbapenems phenotypically either by one or both the tests (Table2). These isolates were also MDR. On genotypic characterization overall the resistance gene was detected in 30/116 (25.86%). Out of which the *bla* NDM gene was more prevalent 18/116 (15. 52%) [Fig. 1 & 2 depict *bla* NDM gene detected at 264bp in 1000bp ladder through PAGE]. As compared to *bla* KPC gene 12/116 (10.34%) (Figs. 3, 4: *bla* KPC gene detected at 782bp in 1000bp ladder through PAGE). Comparison between phenotypic and genotypic tests is shown in Table 3.

Table 1: Isolates of *Klebsiella* species in various clinical samples (n = 336)

S. No.	Clinical Samples	Number of isolates	Percentage
1	Urine	104	30.95%
2	Pus	66	19.64%
3	Sputum	58	17.26%
4	Blood	48	14.29%
5	ET Secretions	38	11.31%
6	Bronchial Wash	10	2.98%
7	Others*	12	3.57%

*Others include Tracheal suction, ET tip, Catheter tip, drain fluid, etc.,

Table 2: Distribution of screen positive and negative isolates of *Klebsiella* spp. Using various phenotypic tests (n = 336)

S. No.	Phenotypic tests	Number	Percentage
1	MHT positive	21	6.25%
2	CDT Positive	44	13.09%
3	MHT & CDT Positive	51	15.18%
4	MHT & CDT Negative	220	65.48%
	Total	336	100%

Table 3: Comparison of genotypic method with the phenotypic tests (n=116)

S. No.	Phenotypic tests	bla NDM gene positive (%)	bla KPC gene positive (%)
1	MHT Positive (n=21)	4 (19.04%)	3 (14.28%)
2	CDT Positive (n=44)	7 (15.90%)	5 (11.36%)
3	Both MHT & CDT Positive (n=51)	7 (13.72%)	4 (13.72%)
	Total positive	**18**	**12**

The chi-square statistic is 0.7613. The p-value is 0.943554. The result is not significant at p< .05.

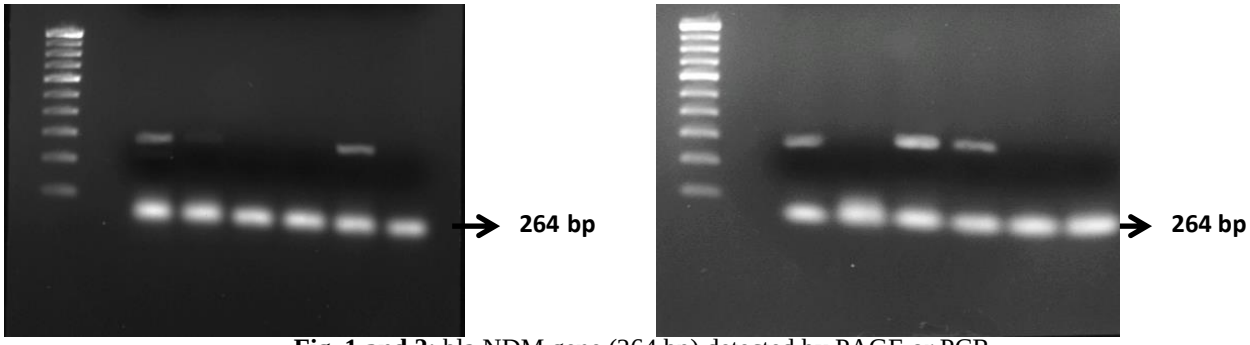

Fig. 1 and 2: bla NDM gene (264 bp) detected by PAGE or PCR

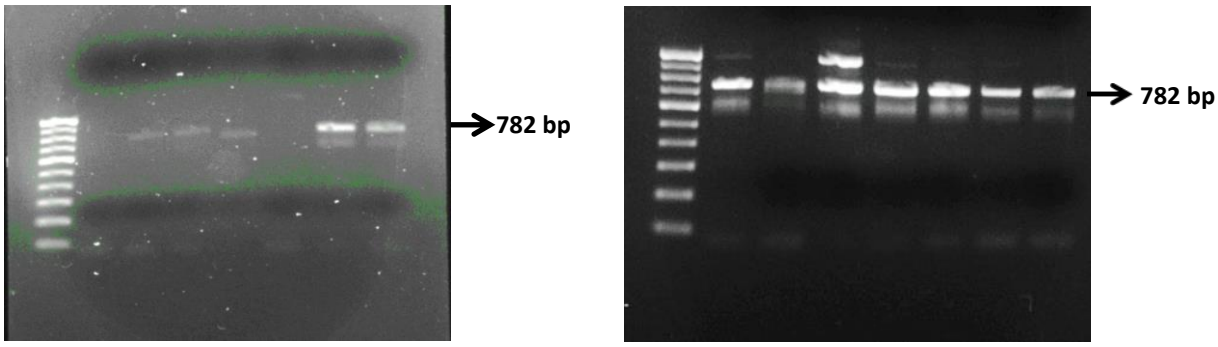

Fig. 3 and 4: bla KPC gene (782 bp) detected by PAGE or PCR

DISCUSSION

The isolation of carbapenemase producing Gram-negative bacilli has increased tremendously during the last few years which is a matter of great concern. Therapeutic options for infections caused by such bacteria expressing carbapenemases are limited. Thus, emphasizing a need to detect carbapenemases harbouring isolates to avoid therapeutic failure and outbreaks.

Klebsiella spp. have higher ability to accumulate and transfer resistant determinants which makes it a leading cause of nosocomial infections. Thus, it becomes important to rapidly detect carbapenemase production in these bacteria and prevent the spread. PCR is considered as the gold standard for testing the genetic resistance in these bacteria. But it is not routinely used due to the high cost of testing and the skills required for the test. Thus, many phenotypic detection methods are used widely for the detection of resistant mechanisms in these bacteria during routine clinical practice. MHT is recommended as an easy and rapid phenotypic method by CLSI 2017 (8). Similarly, CDT is another commonly used phenotypic test to detect carbapenemase production.

The prevalence of carbapenemase producers by phenotypic tests was high (34.52%) in this study as compared to previous similar studies carried out by Chauhan *et al*., (14) and Mohanty *et al*., (15). Increase in the prevalence of carbapenemase producers in the last few years in *Klebsiella* species is a matter of great therapeutic concern. These phenotypic tests are less cumbersome, rapid and cost effective and can be used in routine clinical practice for confirmation of carbapenemase producers where molecular testing is not feasible or possible usually due to limited resources. Similar observation has been reported in an Indian study by Das *et al.,* (16). This would help in

timely initiation of appropriate treatment and would also avoid building further resistance in organisms.

On molecular characterization, the bla NDM gene was the predominant 18 (15.52%) as compared to bla KPC gene 12 (10.34%) in our study. Similar finding of high prevalence of *bla* NDM gene has been reported by other workers (16, 17). NDM and KPC genes are two most prevalent genes among many others that are responsible for carbapenemase production in *Klebsiella* species. This may be due to the fact that the plasmid associated with bla NDM gene has wide rearrangement capabilities and spreads at a high rate in horizontal transmission. However, in many other clinical isolates these 2 genes could not be detected, the reason for this may be due to other genes responsible which were not looked for due to limited resources. This is one of the limitations of our study. In future, the study may be carried out to find out various mechanisms responsible for carbapenem resistance in bacteria.

Klebsiella spp. is a common cause of healthcare associated infections and has been shown to acquire the multidrug resistant genes. Thus, the detection of resistance gene in this bacterium would allow the clinicians to provide appropriate treatment on time and would aid in preventing the spread of multidrug resistant pathogens.

CONCLUSION

MHT and CDT are reliable phenotypic tests to detect carbapenemase producers in routine clinical practice. The presence of bla NDM and blaKPC genes was reported for the first time in Meerut City in Western UP with higher prevalence of bla NDM gene in this region. The presence of such a high rate of antibiotic resistance genes in common pathogens like *Klebsiella* is alarming. In future, the study may be carried out to find out various other mechanisms responsible for carbapenem resistance in bacteria. Timely and accurate detection of resistance would allow the early initiation of treatment for better patient outcome.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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