

**PREVALENCE OF *KLEBSIELLA PNEUMONIAE* CARBAPENEMASE PRODUCTION IN BLOOD CULTURE ISOLATES**

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**ABSTRACT**

**Introduction:** Multidrug resistant (MDR) Gram negative infections have resulted in high rates of morbidity and mortality in patients with diverse clinical conditions. *Klebsiella pneumoniae* carbapenamase (KPC)-producing bacteria is one of the emerging MDR pathogens causing bacteremia with limited therapeutic options such as colistin and tigecycline. **Methods:** An investigational laboratory based study was conducted (over 1 year) to know the prevalence of *Klebsiella pneumoniae* carbapenamase (KPC)-producing bacteria from blood culture isolates. Blood culture samples were processed and *Klebsiella pneumoniae* was identified by colony morphology, Gram staining and biochemical tests, followed by antibiotic susceptibility testing as per CLSI (clinical and laboratory standard institute) guidelines. The isolates were confirmed by automated identification system (BD phoenix). Hundred & ten such isolates were enrolled in the study. The production of carbapenamase was detected by modified Hodge test. **Observations:** The majority of the 110 pts were newborn (62%). Most *Klebsiella pneumoniae* positive blood cultures were submitted from various ICUs (83.6%) followed by burn & plastic surgery department (15.45%). The most sensitive drug for all carbapenamase producing isolates were colistin and tigecycline (100% each). **Conclusions:** MDR KPC was detected to be important organism in NICU and post-surgical patients for enhanced morbidity & mortality. The clinicians are faced with the dilemma as to how to control nosocomial spread of these organism in ICU as there are limited therapeutic options available. Therefore, there is a need to evaluate new detection methods for carbapenamase detection.

**KEYWORDS:** Carbapenamase, *Klebsiella pneumoniae*, drug resistance.**INTRODUCTION**

*K. pneumoniae* has emerged as a potential threat involved in hospital outbreaks, owing to high drug resistance. All the strains are resistant to ampicillin owing to presence of a chromosomal gene encoding penicillin specific  $\beta$ -lactamase.<sup>[1]</sup>

Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage rate and development of multi-drug resistant strains that produce extended-spectrum  $\beta$ -lactamases [ESBL]. During last decade, dissemination of *K. pneumoniae* carbapenamase (KPC) has led to an increase in the prevalence of carbapenam-resistant or pan resistant *Enterobacteriaceae*. These enzymes are also found on mobile genetic elements having the capacity to transfer these genes to other members of the family and frequently encode for resistance to other class of antimicrobials, thereby limiting the choice of antimicrobials left for treatment of such infections. Infection with such strains often lead to complications, increased hospital stay, treatment cost, morbidity and mortality.<sup>[2]</sup>

Unfortunately, from the early 2000s, multidrug-resistant (MDR) *K. pneumoniae* strains started to produce "carbapenemases" encoded by transmissible plasmids and rapidly disseminated within both acute hospitals and long-term care facilities. Later, other enterobacterial species, including *E.coli*, acquired carbapenamase genes, thus suggesting that *K. pneumoniae* may have acted as a pool of  $\beta$ -lactamases. Over the past decade, carbapenemases of Classes A, B and D, which are  $\beta$ -lactamases able to efficiently hydrolyze penicillins, cephalosporins, monobactams, carbapenems and  $\beta$ -lactamase inhibitors, have progressively disseminated among *Enterobacteriaceae*.<sup>[3]</sup>

KPC enzyme-producing *K. pneumoniae* is generally susceptible to few antibiotics, and it is associated with a high mortality rate among patients with bloodstream infections. In fact, many of these strains are susceptible to colistin, tigecycline and one or more aminoglycosides, but some of them are resistant even to these drugs.<sup>[4]</sup>

## MATERIAL AND METHODS

An investigational laboratory based study was conducted in the department of Microbiology, Pt. B.D. Sharma, PGIMS, Rohtak over a period of one year (October, 15 to September, 16). Blood culture samples received in the laboratory during this period were processed initially by standard conventional microbiological techniques. One hundred ten *Klebsiella pneumoniae* isolates were processed further for the purpose of this study and confirmed by automated identification and antibiotic susceptibility testing system (BD phoenix). The antibiotic susceptibility testing was performed as per Clinical and Laboratory Standard Institute Guidelines.<sup>[5]</sup> carbapenamase production detection was done by Modified Hodge Test.

**Biomedical waste:-**All the biomedical waste generated during this study in the laboratory was discarded as per the biomedical waste management and handling rules, 2011 guidelines.<sup>[6]</sup>

**Stastical analysis:-**The data was collected using Microsoft Excel spread sheet and doubly checked for errors. Qualitative data was presented as mean and standard deviation and quantitative data as proportions.

## OBSERVATIONS

This study was conducted on 110 *K. pneumoniae* isolates from blood culture samples over a period of one year i.e. from October, 2015 to September, 2016 in the department of Microbiology of Pt. B. D. Sharma, PGIMS Rohtak. Blood culture samples received in the laboratory during this period were processed initially by standard conventional microbiological techniques. One hundred ten *K. pneumoniae* isolates were processed further for the purpose of this study and were identified on the basis of Gram's staining, colony morphology on blood agar and MacConkey agar, and standard biochemical tests. The antibiotic susceptibility testing was performed as per CLSI guidelines. The isolates were also confirmed by

automated identification and antibiotic susceptibility testing system (BD phoenix).

A total of 15141 samples were received in the department over a period of one year (Oct 2015 – Sep 2016). Out of these, 2301(15.2%) samples were culture positive. Two hundred thirty three (10.12%) *K. pneumoniae* isolates were recovered on processing of cultures by the conventional methods i.e. by studying the colony morphology on the culture plates, gram staining, catalase test and various biochemical reactions as well as automated system.

The age range in the study population in case of females was from newborn to 84 years with mean age 6.62±1.02. The age range in case of males was newborn to 62 with mean age 7.13±2.07. The maximum number of patients i.e. 62 (56.36%) belonged to <1 years age group followed by age group 21-30 years i.e. 13 (11.8%). Only 2 (1.8%) patients found in 1-10 and >60 years age group each. Blood stream *Klebsiella* infections were found to be more common in new born (54.54%).

Out of 110 patients included in the study, *K. pneumoniae* infections were marginally more common in male i.e. 52.7% in comparison to females (47.3%). The ratio of M:F was 1.07:1.

The study reveals that these isolates had varying degree of susceptibility to the antimicrobials tested. All the strains i.e. 100% were sensitive to colistin and tigecycline. Most of these showed high susceptibility to carbapenems group of antibiotics in-vitro viz. imipenem (84%), meropenem (82%) and ertapenem (77%). Least susceptibility of the isolates was seen against ampicillin (5%) followed by gentamicin (11%) and amoxicillin-clavulanic acid (15%). This shows *K. pneumoniae* have developed resistance to most commonly employed antibiotics namely fluoroquinolones, penicillin group, cephalosporins in this tertiary care study.

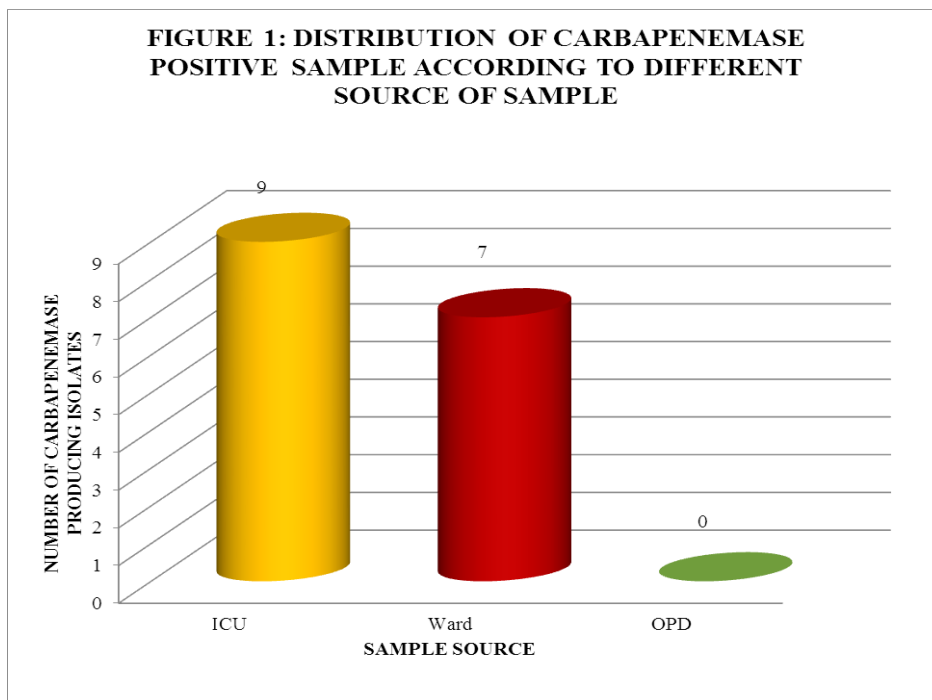
**Table 1: Carbapenamase production among *K. pneumoniae* isolates.**

Total number of <i>K. pneumoniae</i> isolates	Number of isolates producing carbapenamase	Percentage (%)
110	16	14.54

Table 1 shows the carbapenamase production among blood culture isolates of *K. pneumoniae*. It was observed that out of 110 *K. pneumoniae* isolates 16 (14.54%) were carbapenamase producers.

On evaluating the source of samples for carbapenamase producing isolates as shown in figure 1, it was observed

that 100% of these isolates were from hospitalized patients. It was seen that 9 (56.25%) sample out of 16 carbapenamase positive sample were from ICU and 7(43.75%) were from various wards.



**Table 2: Association of carbapenemase production with MDR *K. pneumoniae*.**

Number of carbapenemase producing isolates	MDR isolates among carbapenemase producing isolates	Percentage (%)
16	16	100

Table 2 shows the association of carbapenemase producing strains with that of the multidrug resistant strains. All the carbapenemase producing strains were MDR isolates of *K. pneumoniae*.

**DISCUSSION**

Microbial invasion of the blood stream constitutes one of the most serious condition in the infectious disease. *K. pneumoniae* is among the most common cause of nosocomial gram-negative bacteremia second only to *E. coli* encountered worldwide. According to CDC, 3 to 7% of nosocomial bacterial infections are attributed to *K. pneumoniae*, placing it among eight most important infectious pathogen in hospitals. It is responsible for 4-15% of septicemia in adults and 3-20% of neonatal septicemia (both early and late onset) especially in premature infants and ICU. It accounts for 8% of endemic hospital infections and 3% of epidemic outbreaks, thereby increasing the disease burden, morbidity and mortality.<sup>[7,8]</sup>

Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage rate and development of multi-drug resistant strains that produce extended-spectrum  $\beta$ -lactamases [ESBL]. During last decade, dissemination of *K. pneumoniae* carbapenemase (KPC) has led to an increase in the prevalence of carbapenem-resistant or pan resistant *Enterobacteriaceae*. These enzymes are also found on mobile genetic elements having the capacity to transfer

these genes to other members of the family and frequently encode for resistance to other class of antimicrobials, thereby limiting the choice of antimicrobials left for treatment of such infections. Infection with such strains often lead to complications, increased hospital stay, treatment cost, morbidity and mortality.<sup>[2]</sup>

In the present study, it was observed that out of 110 *K. pneumoniae* isolates 16 (14.54%) were carbapenemase producers. In a cross-sectional study conducted in Egypt by Moemen *et al*<sup>[9]</sup> included all the patients admitted in ICU'S over one year period. 125 *K. pneumoniae* isolates were enrolled for the purpose of study and 42 out of 125 (33.6%) patients had CRKP infection in various samples. CRKP infection among blood isolates was 9.5%. Similarly, Jeniffer *et al*<sup>[10]</sup> who studied epidemiology of carbapenem-resistant *K. pneumoniae* in a network of long-term acute care hospitals found that out of 3846 *Klebsiella pneumoniae* samples from various sites of infection. CRKP was found in 9.4 % of blood culture isolates. CRKP prevalence in the above 2 studies is comparable with present study.

On evaluating the source of samples for carbapenemase producing isolates it was observed that 100% of these isolates were from hospitalized patients. It was seen that 9 (56.25%) sample out of 16 carbapenemase positive sample were from ICU and 7 (43.75%) were from various wards. .QinOu *et al*<sup>[11]</sup> studied prevalence of carbapenem-resistant *K. pneumoniae* (CRKP) in a

hospital in central China and found that 44.4% (52/117) of CRKP strains were isolated from specimen of ICU. In a similar study by Wattal *et al.*,<sup>[12]</sup> who studied surveillance of multidrug resistant organisms in a tertiary care hospital in Delhi reported 51% of CRKP were from ICU's and 31% CRKP from wards. In another study by Nair *et al.*,<sup>[13]</sup> who retrospectively conducted a study in a tertiary care hospital in Mumbai to know the prevalence of CRKP. They included samples from wards, ICU's and outdoor patient department and observed that the majority of the samples which had CRKP isolated from blood were from various ICU's. The admission to the ICU is an independent risk factor for the emergence of carbapenemase producing *K. pneumoniae* infections as well as carriage as reported previously by many epidemiological studies. The finding of the present study are in accordance with the previous studies.<sup>[14]</sup>

Association of carbapenemase producing strains with that of the multidrug resistant strains was studied in present study and it was seen that all the carbapenemase producing strains were MDR isolates of *K. pneumoniae*. Eshetie *et al.*<sup>[15]</sup> reported overall prevalence of CPE as 2.73% and all CPE strains were 100% ESBL producer and completely resistant to ampicillin, cefotaxime, cefpodoxime, co-trimoxazole, chloramphenicol, and amoxicillin-clavulanic acid. Kumar *et al.*<sup>[13]</sup> studied leading trend of carbapenem resistance in *Enterobacteriaceae* in India and found that all CRKP were resistant to more than 3 categories of drugs i.e. multidrug resistant which is in agreement with the present study.

Therefore, it can be concluded that *K. pneumoniae* is a potential threat as it is a cause of numerous hospital acquired as well as community acquired infections, owing to its ability to produce various virulence factors and development of antibiotic resistance to various classes of drug including carbapenem. Considering the overall scenario in a developing country like ours, there is a need to evaluate the available test for carbapenemase detection for early and accurate detection. This shall result in appropriately guiding the treating physicians in use of antibiotics which in turn might impact hospital outcome of the patients and thereby decreasing morbidity and mortality. However, being a small study it is recommended that the data to be extrapolated only after large population based trials.

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**CONFLICT OF INTEREST:** NIL.

## REFERENCES

1. Donnenberg MS. Enterobacteriaceae. In: Bennet JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's principles and practices of infectious diseases. 8<sup>th</sup> ed. Canada: Elsevier; 2015: 2503-17.
2. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis.*, 2011 Mar; 53(1): 60-7.
3. Patel G, Bonomo R.A. Status report on carbapenemases: challenges and prospects. *Expert Rev. Anti Infect. Ther.*, 2011 May; 9(5): 555-70.
4. Wang, Q.; Li, B.; Tsang, A.K.; Yi, Y.; Woo, P.C.; Liu, C.H. Genotypic analysis of *Klebsiella pneumoniae* isolates in a Beijing hospital reveals high genetic diversity and clonal population structure of drug-resistant isolates. *PLoS One*, 2013 Feb, 8: e57091.
5. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twenty-Seventh informational supplement. CLSI document M100-S27. Wayne, PA: CLSI, 2017.
6. moef.nic.in[Internet]. New Delhi; Govt. of India, Ministry of Environment and Forests: draft biomedical waste (management and handling) rules, 2011; [cited 2011]. Available from: <http://www.moef.nic.in/downloads/public-information/salient-features-draft-bmwmh.pdf>. [Accessed on 10 Aug, 2015].
7. Abhilash KPP, Veeraraghavan B, Abraham OC. Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella spp.* in a tertiary care hospital in south India. *JAPI.*, 2010 Dec; 58: 13-7.
8. Podschun R, Ullmann U. *Klebsiella spp.* as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev*, 1998 Oct; 11(4): 589-603.
9. Moemen D, Masallat D. Prevalence and characterization of carbapenem-resistant *Klebsiella pneumoniae* isolated from intensive care units of Mansoura University hospitals Egypt *J Bas Appl Sci.*, 2017 Jan; 37-41.
10. Han JH, Goldstein EJ, Wise J, Bilker WB, Tolomeo P, Lautenbach E. Epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in a network of long-term acute care hospitals. *Clin Infect Dis.*, 2017 Apr; 64(7): 839-44.
11. Ou Q, Li W, Li B, Yu C. Prevalence of Carbapenem-Resistant *Klebsiella Pneumoniae* (CRKP) and the Distribution of Class 1 Integron in Their Strains Isolated from a Hospital in Central China. *Chin Med Sci J.*, 2017 Jun 10; 32(2): 107-2.
12. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Surveillance of multidrug resistant organisms in a tertiary care hospital in Delhi. *J Assoc Physicians India*, 2010 Dec; 58 Suppl: 32-6.
13. Nair P. K, et al. Carbapenem resistant *Enterobacteriaceae* from a tertiary hospital. *Microbiol Infect Dis*, 2013 Dec; 3(4): 207-10.
14. Kang CI, Kim SH, Park WB et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on

- outcome. *Antimicrob Agents Chemother*, 2005 May; 49: 760–766.
15. Eshetie S, Unakal C, Gelaw A, Ayelign B, Endris M, Moges F. Multidrug resistant and carbapenemase producing *Enterobacteriaceae* among patients with urinary tract infection at referral hospital, northwest Ethiopia. *Antimicrob Resist Infect Control*, 2015; 4: 12.