



Original Research Article

Prevalence of *Klebsiella pneumoniae* Carbapenemase (KPC), Metallo Beta Lactamases and AmpC beta Lactamases in Clinical Isolates of *Klebsiella* Species

Shalley Dahiya^{1*}, Pooja Singla², Uma Chaudhary³ and Bijender Singh⁴

¹Department of Microbiology, BPS govt. medical college for women, Khanpur Kalan, Sonapat, Haryana, India

²Department of Microbiology, SHKM govt. medical college, Mewat, Haryana, India

³Department of Microbiology, PT. B.D.Sharma PGIMS Rohtak, India

⁴Medical officer, HCMS, India

*Corresponding author

ABSTRACT

The emergence of carbapenem-resistant *Klebsiella* spp. due to presence of *Klebsiella pneumoniae* carbapenemase (KPC), metallo-beta-lactamase (MBL) and AmpC with porin loss is of great concern. The aim of the study is to know prevalence of KPC, MBL and AmpC in clinical isolates of *Klebsiella* spp. in our hospital. This prospective study was carried out in the Department of Microbiology, at our tertiary care hospital, North India, from July 2012 to June 2013. All isolates of *Klebsiella* spp. were isolated from various clinical samples and identified according to standard Microbiological procedures. Resistance to ertapenem (10µg)/ meropenem (10µg) was detected by disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) criteria. Phenylboronic acid (PBA), dipicolinic acid and cloxacillin along with meropenem and meropenem disc alone was used for detection of KPC, MBL and AmpC respectively. Out of 861 strains of *Klebsiella* spp., 127 strains were resistant to carbapenem (prevalence rate 14.75%). None of the isolates were KPC and AmpC with porin loss producers, 31 isolates were MBL producers (3.6%). With the emergence of multi-pronged resistance mechanisms in clinical isolates, accurate and timely detection of resistance mechanisms is important for infection control and better patient outcomes.

Keywords

Klebsiella pneumoniae
Carbapenemase (KPC),
Metallo
Beta Lactamases
and
AmpC beta
Lactamases

Introduction

Carbapenems are chosen as first line drugs for severe infections caused by *Enterobacteriaceae* expressing extended spectrum beta-lactamases (ESBLs). Unfortunately, bacterial beta-lactamases significantly hamper the efficacy of these life-saving antibiotics. Carbapenemases are

beta-lactamases with versatile hydrolytic capacities. They have the ability to hydrolyse penicillins, cephalosporins, monobactams and carbapenems. Carbapenemases are members of the molecular class A, B, and D beta-lactamases. The class A carbapenemase

group includes members of the *Serratia marcescens* enzyme (SME), imipenem hydrolysing beta-lactamase (IMI), not metallo enzyme carbapenemase (NMC), Guiana extended spectrum (GES) and KPC families. Of these, the KPC carbapenemases are the most prevalent, found mostly on plasmids in *K. pneumoniae*. The class D carbapenemases consist of OXA type beta-lactamases frequently detected in *Acinetobacter baumannii*. The metallo-beta-lactamases have been detected primarily in *Pseudomonas aeruginosa*; however, there is increasing number of reports worldwide of this group of beta-lactamases in the *Enterobacteriaceae* (Queenan and Bush, 2007). Several phenotypic tests for the detection of KPCs have been developed. The method currently advised by the Clinical and Laboratory Standard Institute (CLSI) is the modified Hodge test (MHT) which has acceptable sensitivity and specificity for carbapenemase production. However, its interpretation can be difficult for some isolates and false positives have been reported.

A second phenotypic method shown to be promising for identification of KPCs utilizes boronic acid (BA) based compounds. BA was originally described in the 1980s as a reversible inhibitor of class C beta-lactamases and has been used in combination disc tests for the identification of AmpC producing isolates. Recently, several disc tests combining BA compounds, phenylboronic acid and 3-aminophenyl boronic acid (APB), have been proved to be highly sensitive and specific for the detection of KPC production (Hirsch and Tam, 2010). Several inhibitor-based tests have been developed for the specific detection of MBL producers, which are based on the synergy between MBL inhibitors such as EDTA, EDTA plus 1,10-phenanthroline, thiol compounds and dipicolinic acid and a carbapenem and/or an

oxymino cephalosporin as indicator beta lactam compounds (Miriagou *et al.*, 2010). The detection of KPC, MBL and AmpC hyperproducers can be detected easily using these inhibitors by disc diffusion method as recommended by a group of experts from EUCAST and the ESCMID Study Group for Antibiotic Resistance Surveillance (EARSS) (Tsakris *et al.*, 2009; Beesley *et al.*, 1983). The emergence of carbapenem-resistant *Klebsiella* due to KPC, MBL and AmpC with porin loss, in the past years is of great concern. With increasing resistance and therapeutic failure in hospitalised patients, there is a need to screen the clinical isolates for resistance mechanisms as it will impact decision for appropriate choice of antibiotics.

Materials and Methods

Sample collection: The present prospective study was conducted in the Department of Microbiology at a tertiary level teaching health care facility from July 2012 to June 2013. One hundred non-duplicate *Klebsiella* isolates, recovered from urine, pus, blood, respiratory samples, CSF, high vaginal swabs and various body fluids were included in the study. The clinical specimens were collected from both indoor and outdoor patients irrespective of age and gender.

Isolation, identification and antimicrobial susceptibility testing of *Klebsiella* species: For the isolation of *Klebsiella* spp., the samples were inoculated onto blood agar and MacConkey agar and incubated at 37°C for 24 hrs. The suspected colonies were further processed for identification by Gram staining, oxidase test, and other standard biochemical tests. The isolates of *Klebsiella* species were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method using CLSI criteria on Mueller-Hinton agar (MHA) (CLSI document, 2006). Antibiotic discs used in

the study were procured from Hi-media[®] Laboratories, Mumbai, India and from BD diagnostics[®] USA. American Type Culture Collection (ATCC) strain viz. *E. coli*. ATCC 25922 was employed as a control strain (Koneman's color Atlas and Textbook of Diagnostic Microbiology, 6th edn). Discs of the following antimicrobial agents, with their disc concentration, in brackets, were used: Ampicillin (10µg), gentamicin (10µg), amikacin (30µg), amoxicillin / clavulanic acid (20µg/10µg), ampicillin/sulbactam (10µg/10µg), piperacillin / tazobactam (100µg/10µg), ticarcillin / clavulanic acid (75µg/10µg), cefuroxime (30µg), cefepime (30µg), cefotaxime(30µg), ceftriaxone (30µg), ciprofloxacin (5µg), levofloxacin (5µg), ertapenem (10µg), imipenem (10µg), meropenem (10µg), piperacillin (100µg), trimethoprim/sulfamethoxazole (1.25µg/23.75µg), aztreonam (30µg), ceftazidime (30µg). For urinary isolates, ofloxacin (5µg), norfloxacin (10µg), and nitrofurantoin (300µg) were also tested.

Detection of carbapenemases

KPC/MBL confirm kit (Rosco Diagnostica, Denmark)

The kit consisted of four cartridges containing 50 Neo-Sensitabs (50 tests) with recognisable and distinguishable codes: Meropenem 10 µg (MRP10); Meropenem 10 µg + Boronic acid (MRPBO); Meropenem 10µg + Cloxacillin (MRPCX) and Meropenem 10µg + Dipicolinic acid (MRPDP). Cartridges were allowed to acclimatise to room temperature for 30–60 minutes before the lid was removed from the cartridge. A suspension of the organism to be tested equivalent to McFarland 0.5 was spreaded uniformly over the entire area of a Mueller Hinton susceptibility agar plate. Using a single disc dispenser, each disc was placed on the inoculated agar plate, ensuring sufficient space between individual discs to

allow for proper measurement of inhibition zones. The plate was incubated at 37°C for overnight. The diameter of the inhibition zones were measured and recorded. No zone around a disc corresponded to a nine mm inhibition zone. The interpretation of carbapenemase by kit is depicted in table 1 and figure 1.

Statistical analysis

At the end of the study, results were collected and analysed by using SPSS software version 17.0. If the p value was <0.05, it was considered significant.

Results and Discussion

A total of 861 non duplicate, non consecutive *Klebsiella* isolates were processed for species identification and antimicrobial susceptibility testing. *K. pneumoniae* was the commonest species isolated 760/861(88%), followed by *K. oxytoca* 101/861(12%). Out of 861 strains of *Klebsiella* spp., 127 strains were resistant to carbapenems (14.75%). Maximum carbapenem resistant isolates were from pus samples (21.55%) followed by throat swabs (18.42%), urine (15.59%), stool (9.37%), blood (9.18%), HVS (7.89%), lower respiratory tract samples (7.69%) and body fluids (4%). The male to female ratio among patients with *Klebsiella* infection was 1.5:1. And majority of the patients belonged to age group 21-30 years (32%), followed by age group 41-50 years (20%) and by age group more than 60 years (13%). Randomly selected hundred isolates showing reduced susceptibility to carbapenems and third generation cephalosporins were further processed for detection of various carbapenemases using phenylboronic acid (PBA), dipicolinic acid and cloxacillin along with meropenem and meropenem disc alone. None of the isolates were KPC and AmpC with porin loss producers, 31 isolates were

MBL producers (3.6%). Maximum MBL production was seen in urinary isolates (32.3%), followed by pus (29%), ETI (16.1%), blood (9.7%), sputum (9.7%) and stool (3.2%) (Table 2). On comparing the resistance among MBL producing and non MBL producing *Klebsiella* isolates, significant difference in terms of p value was observed for ampicillin-sulbactam, piperacillin tazobactam and ceftriaxone (p value <0.05). When resistance to aminoglycosides was compared among MBL producing and non MBL producing *Klebsiella* isolates, p value was found to be <0.05 for amikacin and gentamicin which indicated significant difference (Figure 2).

Klebsiella has been associated with different types of infections and one of the important aspects of *Klebsiella* associated infections is the emergence of multidrug resistant strains particularly those involved in nosocomial diseases. In the current study, the rate of isolation of *Klebsiella* species from the culture positive specimens was 8.95%. The isolation rate of *Klebsiella* spp. from various other studies ranged from 9.3% to 25%. (Sarathbabu *et al.*, 2012; Soltan *et al.*, 2013; Acheampong, 2011).

This variation can be attributed to varying rates of prevalence of *Klebsiella* spp. in environment and community in different geographical areas. In the present study, maximum carbapenem resistant isolates were identified from pus samples (21.55%), followed by throat swabs (18.42%), urine (15.59%), stool (9.37%), blood (9.18%), HVS (7.89%), LRTS (7.84%) and body fluids (4%).

However, Rai *et al.* (2011) reported that out of 102 isolates of carbapenem resistant *Klebsiella* isolates, 89.2% were from urine samples and 10.78% were from blood. Parveen *et al.* (2010) also reported a higher percentage of carbapenem resistant

Klebsiella from urine samples (40%) followed by blood (20%), wound discharge (13.3%), peritoneal fluid (6.67%), ascitic fluid (11.11%), tracheal aspirate (4.4%), and sputum (4.4%).

In the present study, among carbapenems, 91% isolates of *Klebsiella* spp. were resistant to imipenem. The results of present study were in accordance with Parveen *et al.* (2010) who reported that among meropenem resistant *Klebsiella* isolates, 73.3% were also resistant to imipenem. Shah *et al.* also reported 86.96% resistance to imipenem (Shah and Desai, 2012). As the use of carbapenems to treat infections varies from hospital to hospital, so the level of resistance may vary from hospital to hospital.

In the current study the isolates were also tested by KPC+MBL detection kit. None of the isolates were detected to be KPC producer by using boronic acid as inhibitor. Thirty one isolates were found to be MBL producer using dipicolinic acid. None of the isolates were detected to be AmpC producer by using cloxacillin as inhibitor. This was in concordance with study by Datta *et al.* (2012) who reported 75% (9/12) isolates of *Klebsiella* to be MBL producer. None of the isolates were KPC or AmpC producer using boronic acid and cloxacillin as inhibitor. However, Ambretti *et al.* (2013) identified fifty isolates of *Klebsiella pneumoniae* producing KPC by using boronic acid as inhibitor, seven isolates producing MBL using dipicolinic acid as inhibitor and one isolate producing AmpC using cloxacillin as inhibitor.

Higher prevalence of KPC producing isolates of *Klebsiella* was reported by Gupta *et al.* They reported that out of 41 carbapenem resistant *Klebsiella*, 28 isolates were positive by MHT. One isolate was found to be positive for AmpC using cloxacillin as inhibitor.

Table.1 Interpretation of results by comparing the inhibition zones of the different discs

		Meropenem and boronic acid	Meropenem and dipicolinic acid	Meropenem and cloxacillin
AmpC+porin loss	Meropenem 10 µg	≥5 mm	<5 mm	≥5 mm
KPC	Meropenem 10 µg	≥5 mm	<5 mm	<5 mm
MBL	Meropenem 10 µg	<5 mm	≥5 mm	<5 mm

Table.2 Distribution of metallo beta-lactamase (MBL) producing *Klebsiella* in different clinical isolates

Name of specimen	Total number of isolates	MBL producing isolates	Percentage (%)
Blood	9	3	9.7
Drain	1	0	-
ETI	17	5	16.1
HVS	3	0	-
Pus	29	9	29
Sputum	7	3	9.7
Stool	6	1	3.2
Urine	28	10	32.3
Total	100	31	31

Figure.1 Detection of carbapenemases by kit method

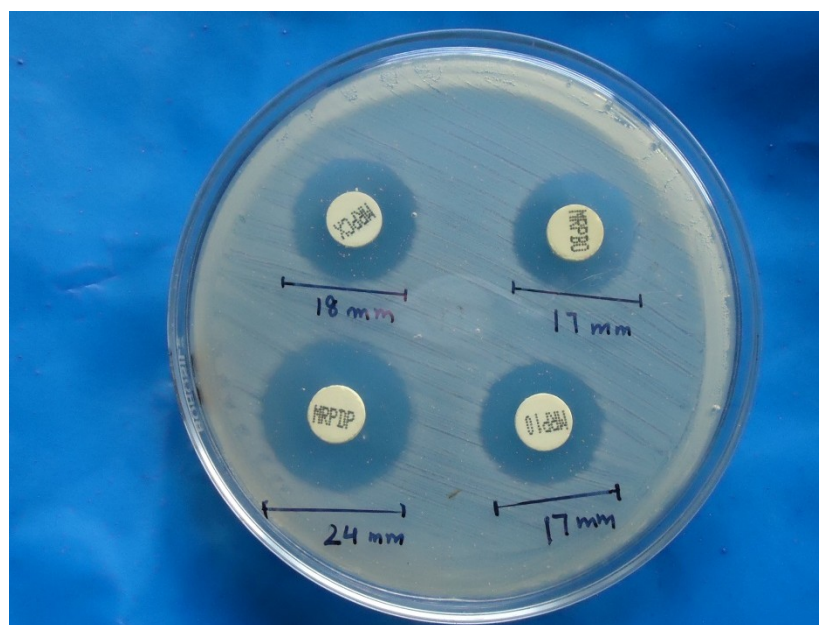
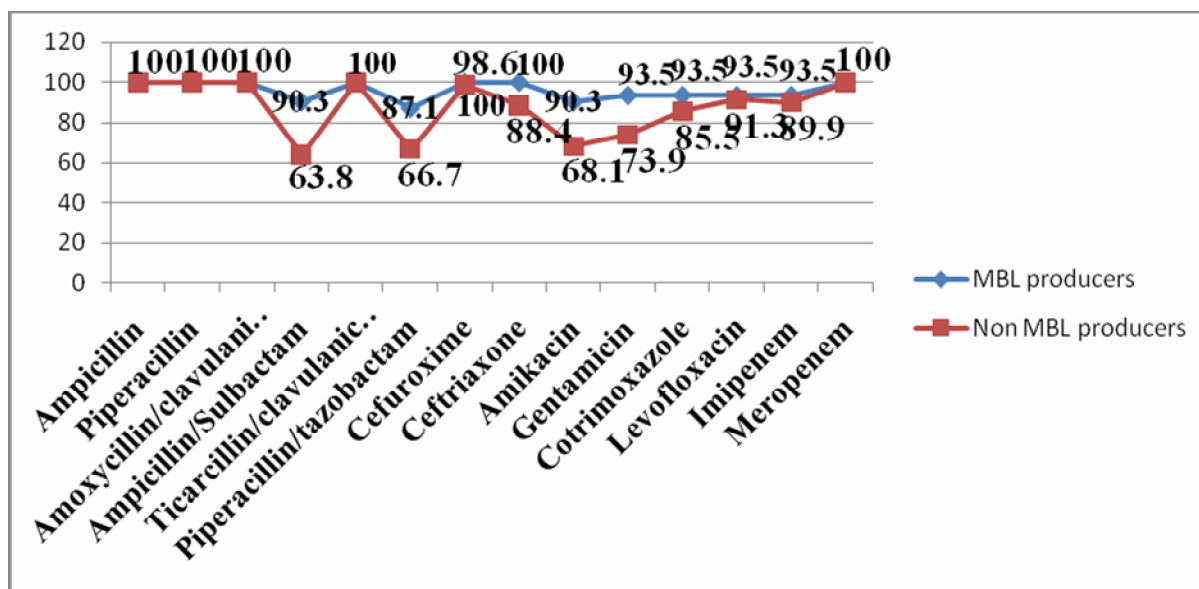


Figure.2 Comparison of the antimicrobial resistance pattern of MBL producing and non MBL producing *Klebsiella* isolates



Twenty five isolates were identified to be KPC producer by using boronic acid as inhibitor (Gupta *et al.*, 2013). Tsakris *et al.* (2009) tested discs containing 400 µg of phenylboronic acid as an inhibitor and several beta-lactams as the antibiotic substrates against 57 KPC- producing isolates. They found significantly increased (>5mm) inhibition zone diameters when used in combination with cefepime and all carbapenems (imipenem, meropenem and ertapenem) compared with zones produced by the beta-lactam discs alone. Doi *et al* found that the addition of APB to ertapenem or meropenem (but not imipenem) discs resulted in an increased zone diameter ≥ 5 mm for ten KPC producing isolates when compared with the carbapenem disc alone (Doi *et al.*, 2008).

In many healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming rampant, complicating treatment and increasing both human morbidity and financial costs. This necessitates the need for detecting the resistant bacteria so that

unnecessary use of broad spectrum antimicrobials can be avoided. In the present study carbapenem isolates also showed significant coresistance to other class of antibiotics thus, leaving behind only few therapeutic options. Failure to detect carbapenemases has contributed to their uncontrolled spread and therapeutic failures. So, these carbapenemases should be detected routinely in clinical laboratories by using appropriate methods and reported to clinicians at time so that inappropriate use of antibiotics is avoided.

References

- Acheampong, D.O., Boamponsem, L.K., Feglo, P.K. 2011. Occurrence and species distribution of *Klebsiella* isolates: A case study at Komfo Anokye teaching hospital (Kath) in Ghana. *Adv. Appl. Sci. Res.*, 2: 187–193.
- Ambretti, S., Gaibani, P., Berlingeri, A., Cordovana, M., Tamburini, M.V., Bua, G. 2013. Evaluation of phenotypic and genotypic approaches for the detection of class A and class B carbapenemases in

- Enterobacteriaceae. Microb. Drug Resist.*, 19: 212–215.
- Beesley, T., Gascoyne, N., Knott-Hunziker, V. 1983. The inhibition of class C beta-lactamases by boronic acids. *Biochem. J.*, 209: 229–233.
- Clinical and Laboratory Standards Institute (CLSI). 2006. Performance standards for antimicrobial disk susceptibility tests: approved standard, 9th edn. CLSI document M2-A9, Vol. 26(1). Wayne, PA.
- Datta, P., Gupta, V., Garg, S., Chander, J. 2012. Phenotypic method for differentiation of carbapenemases in *Enterobacteriaceae*: Study from north India. *Indian J. Pathol. Microbiol.*, 55: 357–360.
- Doi, Y., Potoski, BA., Adams-Haduch, JM. 2008. Simple disc-based method for detection of *Klebsiella pneumoniae* carbapenemase-type beta-lactamase by use of a boronic acid compound. *J. Clin. Microbiol.*, 46: 4083–4086.
- Gupta, V., Bansal, N., Singla, N., Chander, J. 2013. Occurrence and phenotypic detection of class A carbapenemases among *Escherichia coli* and *Klebsiella pneumoniae* blood isolates at a tertiary care center. *J. Microbiol. Immunol. Infect.*, 46: 104–108.
- Hirsch, EB., Tam, V.H. 2010. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug resistant infection. *J. Antimicrob. Chemother.*, 65: 1119–1125.
- Miriagou, V., Cornaglia, G., Edelstein, M., Galani, I., Giske, C.G., Gniadkowski, M. 2010. Acquired carbapenemases in gram-negative bacterial pathogens: detection and surveillance issues. *Clin. Microbiol. Infect.*, 16: 112–122.
- Parveen, R.M., Harish, B.N., Parija, S.C. 2010. Emerging carbapenem resistance among nosocomial isolates of *Klebsiella pneumoniae* in south India. *Int. J. Pharm. Bio Sci.*, 1: 1–11.
- Queenan, A.M., Bush, K. 2007. Carbapenemases: the versatile beta lactamases. *Clin. Microbiol. Rev.*, 20: 440–58.
- Rai, S., Manchanda, V., Singh, N.P., Kaur, I.R. 2011. Zinc-dependent carbapenemases in clinical isolates of family *Enterobacteriaceae*. *Indian J. Med. Microbiol.*, 29: 275–9.
- Rosco diagnostic, 2011. KPC and MBL confirm kit detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™. <http://www.rosco.dk/default.asp?mainmenu=3&submenu=61&webmanage=KPC%20and%20MBL%20Confirm%20Kit> (Accessed 3 Dec. 2011).
- Sarathbabu, R., Ramani, T.V., Bhaskara, K., Panda, S. 2012. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. *J. Pharm. Bio Sci.*, 1: 4–9.
- Shah, M.N., Desai, P.B. 2012. Emergence of multidrug-resistant *Klebsiella pneumoniae* in neonatal intensive care units: concern about antimicrobial policies. *Res. J. Recent Sci.*, 1: 275–280.
- Soltan, M.M., Sharifi Yazdi, M.K., Avadisians, S., Agha Mirazaei, H., Sabaghi, A. 2013. Efficacy of ciprofloxacin, ceftizoxims and carbenicillin on *Klebsiella* species isolated from hospital specimens. *J. Gorgan. Uni. Med. Sci.*, 15: 77–83.
- Tsakris, A., Kristo, I., Poulou, A. 2009. Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumoniae* isolates in the clinical laboratory. *J. Clin. Microbiol.*, 47: 362–367.
- Winn Jr. W.C., Allen, S.D., Janda, W.M., Koneman, E.W., Procop, G.W., Schreckenberger, P.C., et al. (Eds). 2006. Antimicrobial susceptibility testing. Koneman's color atlas and textbook of diagnostic microbiology, 6th edn. Lippincott Williams and Wilkins, Philadelphia. Pp. 946–1014.