PREVALENCE OF K. PNEUMONIAE CARBAPENEMASE (KPC) AND METALLOBETA-LACTAMASE (MBL) IN URINARY ISOLATES OF KLEBSIELLA SPECIES IN A TERTIARY HEALTH CARE FACILITY IN NORTHERN INDIA

Dr. Shalley Dahiya*1, Dr. Bijender Singh2, Dr. Pooja Singla3, Dr. Uma Chaudhary4

1Medical Officer, Department of Microbiology, BPS Govt. Medical College for Women, Khanpur Kalan, Sonepat, Haryana.
2Medical officer, HCMS.
3Assistant Professor, Department of Microbiology, SHKM Govt. Medical College, Mewat, Haryana.
4Sr. Prof and Head, Department of Microbiology, PT. B.D.Sharma PGIMS Rohtak.

ABSTRACT

Introduction: *Klebsiella* spp. is an important nosocomial pathogen of urinary tract infections. The treatment options are narrowing down because of multidrug resistance (MDR) nature of this organism due to the production of the versatile genetically mobile metallo-beta-lactamases (MBLs) and *Klebsiella pneumoniae* carbapenemases (KPC). Carbapenems are the mainstay of these MDR isolates but in recent times increasing resistance to carbapenems is being reported. This calls for an accurate drug susceptibility testing so that effective therapeutic interventions can be done. Objectives: To study the prevalence of carbapenem resistance and detection of KPC and MBL in urinary isolates of *Klebsiella* spp. in our hospital. Materials and methods: A total of 186 isolates from urine samples from various OPDs and wards of PGIMS Rohtak during the period of July 2012 to June 2013 were included in the study. All the isolates were identified according to standard Microbiological procedures and antimicrobial susceptibility testing was done by Kirby-Bauer’s disc diffusion method as per Clinical Laboratory Standard Institute (CLSI) criteria. The isolates that were resistant to Ertapenem/Imipenem were screened for MBL production by Imipenem+EDTA and KPC
production by Meropenem+Boronic acid and modified Hodge test respectively. **Results:** Out of 186 isolates of *Klebsiella* spp., 28 isolates (26 *K.pneumoniae*, 2 *K.oxytoca*) were resistant to carbapenem. Ten isolates were positive for MBL production and none of the isolates was KPC producer. **Conclusion:** The frequent use of carbapenems has led to an increase in MDR isolates resulting in limited therapeutic options. It is therefore mandatory to detect the MBL and KPC producers by simple and cost constraint methods for planning and implementing effective infection control measures.

**KEYWORDS:** Klebsiella, metallo beta lactamases, modified Hodge test, *Klebsiella pneumoniae* carbapenemases, multidrug resistance.

**INTRODUCTION**

Urinary tract infections (UTIs) are one of the most common infections to be encountered in clinical practice.\(^1\)\(^-\)\(^3\) They may be symptomatic or asymptomatic, and either type of infection can result in serious complications if not treated appropriately.\(^4\) Although different causative agents can be responsible for UTIs, bacteria are the major cause being responsible for more than 95% of UTI cases.\(^5\) *Klebsiella* spp. have been identified as an important common pathogen for nosocomial urinary tract infections (6 to 17%).\(^6\) Owing to its ability to produce extended spectrum beta lactamases (ESBL), carbapenems have become the preferred antimicrobials for treating such conditions which in turn has resulted in emergence of carbapenem resistant strains.\(^7\) Resistance to carbapenems is mostly due to production of enzymes carbapenemases, which are divided into Ambler Classes A, B and D. Class A (serine carbapenemase) enzymes include enzymes such as KPC, IMI, SME, etc, and are commonly present in members of *Enterobacteriaceae*.\(^8\) Many of the microorganisms harbour carbapenemase (KPC) or CTX-M β-lactamase (CTX) enzymes, which confer resistance to various classes of antibiotics. A substantial proportion of isolates produce both enzymes or have other resistance mechanisms. Therefore, we conducted this prospective study to evaluate the prevalence of carbapenem resistance in *Klebsiella* spp. isolated from clinical specimens.

**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 eighty six non-duplicate *Klebsiella* isolates, recovered from urine sample 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).[^9]

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.[^10] 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), ofloxacin (5µg), norfloxacin (10µg), and nitrofurantoin (300µg).

**Detection of carbapenemases**

The isolates showing reduced susceptibility to third generation cephalosporins and carbapenemases were tested for KPC detection using following two methods.

**1. Modified Hodge test (MHT)**[^11]

This was done as per CLSI guidelines. MHA plate was examined for enhanced growth around the test or quality control organism streak at the intersection of the streak and the zone of inhibition. As positive quality control KPC positive *K. pneumoniae* ATCC BAA-1705 strain was used. The plate was examined for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disc. (Figure 1 and 2).

- MHT positive test showed a clover leaf-like indentation of the *E. coli* 25922 growing along the test organism growth streak within the disc diffusion zone.
• MHT negative test has no growth of the *E. coli* 25922 along the test organism growth streak within the disc diffusion zone.

2. **KPC/MBL confirm kit (Rosco Diagnostica A/S, Taastrup, Denmark)**[12]

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 10 µg + Cloxacillin (MRPCX) and Meropenem 10 µg + 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. 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 3.

3. **Imipenem-EDTA method for MBL detection**

Isolates showing reduced susceptibility to imipenem were further processed for MBL production by zone enhancement with EDTA impregnated imipenem discs. Test organism was inoculated on MHA plate as per CLSI guidelines. A 0.5M EDTA solution was prepared. Two 10µg imipenem discs were placed on the surface of agar plate and EDTA solution was added to one of them to obtain a desired concentration of 750 µg. The inhibition zones of imipenem and imipenem EDTA discs were compared after 16-18 hours of incubation at 35°C. A positive test was indicated by zone enhancement with EDTA impregnated imipenem. The zone size enhancement of ≥7mm for imipenem EDTA disc as compared to imipenem alone was taken as positive criteria for MBL production (figure 4).[13]

**Data collection and statistical analysis**

At the end of the study, results were collected and analysed by using SPSS software version 17.0. Data were presented as percentages and proportions.

**RESULTS**

A total of 2364 non duplicate, non-consecutive urinary isolates were processed for species identification and antimicrobial susceptibility testing, out of which one hundred eighty six isolates of *Klebsiella* spp. were identified, 7.86% being the rate of isolation. Out of 186
isolates of Klebsiella spp., 133 isolates were of K. pneumoniae and 53 were of K. oxytoca. Twenty eight isolates (26 K. pneumoniae, 2 K. oxytoca) were resistant to carbapenems. All the isolates showed 100% resistance to ampicillin, 32.25% to amoxicillin-clavulanic acid, 37.2% to gentamicin, 18.8% to amikacin, 67.2% to cotrimoxazole, 67.2% to ceftazidime, 17.2% to meropenem and 22% to piperacillin-tazobactam (Figure 5). Among all carbapenem resistant isolates of Klebsiella, twenty four (85.7%) isolates showed resistance to nitrofurantoin and all isolates showed resistance to norfloxacin and ofloxacin. Ten isolates were positive for MBL production and none of the isolates was KPC producing.

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

<table>
<thead>
<tr>
<th></th>
<th>Meropenem and boronic acid</th>
<th>Meropenem and dipicolinic acid</th>
<th>Meropenem and cloxacillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmpC+porin loss</td>
<td>Meropenem 10 µg</td>
<td>≥5 mm</td>
<td>&lt;5 mm</td>
</tr>
<tr>
<td>KPC</td>
<td>Meropenem 10 µg</td>
<td>≥5 mm</td>
<td>&lt;5 mm</td>
</tr>
<tr>
<td>MBL</td>
<td>Meropenem 10 µg</td>
<td>&lt;5 mm</td>
<td>≥5 mm</td>
</tr>
</tbody>
</table>

Figure 1: Modified Hodge test showing positive results

Figure 2: Modified Hodge test showing negative results
Figure 3: Detection of resistance mechanisms by KPC/MBL confirm kit by Rosco

Figure 4: MBL detection by Imipenem – EDTA method

Figure 5: Antimicrobial sensitivity pattern of urinary isolates of *Klebsiella* spp.
DISCUSSION

*Klebsiella* is the most common causative agent of nosocomial and community acquired infections.\(^\text{[14]}\) In the hospital environment, under heavy antibiotic usage, multiple drug resistance has been increasingly observed in *Klebsiella*. The strains harbouring ESBL and more recently resistance of *K. pneumoniae* against carbapenem, imparted by the presence of carbapenemase, is an emerging global health problem with high morbidity and mortality.\(^\text{[15]}\) These multidrug-resistant organisms are affecting the choice of antimicrobial therapy and are a major cause for increasing hospital costs and duration of hospitalisations. In the current study, the rate of isolation of *Klebsiella* species from the culture positive specimens was 7.86% which was lower than the study by Acheampong et al and Akingbade et al who reported an isolation rate of 10.7% and 14% respectively from all the culture positive samples.\(^\text{[16,17]}\) Sarathbabu et al reported 24.36% rate of isolation of *K. pneumoniae* from urine samples.\(^\text{[18]}\) The lower rate of isolation may be due to implementation of better infection control measures in our hospital. Among isolates of *K. pneumoniae* and *K. oxytoca*, resistance against cefotaxime, ceftriaxone, cefepime and ceftazidime was observed in all strains. Other studies on *Klebsiella* have depicted similar results with respect to these antibiotics. Kumar et al reported a high rate of resistance in *Klebsiella* isolates to cefuroxime (77.4%), ceftizoxime (100%), cefepime (66.8%), cefadroxyl (88.8%), ceftazidime (45.1%), cefotaxime (88.8%) and cefoperazone (77.4%) respectively.\(^\text{[19]}\) Similarly, Parveen et al also reported hundred percent resistance to all third and fourth generation of cephalosporins.\(^\text{[20]}\) The study conducted by Ullah et al showed that a total of 54.3% *Klebsiella* isolates were resistant to the third generation cephalosporins (ceftazidime and cefotaxime).\(^\text{[21]}\) This discordance may be due to greater use of cephalosporins for empirical therapy in almost all the patients admitted in our institute. In the current study, isolates identified to be carbapenem resistant were 15.05%. Gupta et al from Delhi reported 6.9% of meropenem resistance and 4.3% of imipenem resistance in *Klebsiella* and a study from Kanpur reported no carbapenem resistance among *K. pneumoniae* tested.\(^\text{[22,23]}\) However a higher rate was reported by Parveen et al, who reported resistance to meropenem in 43.65% isolates of *Klebsiella* spp. in 2010.\(^\text{[20]}\) In the present study carbapenemases were screened by modified Hodge test as per CLSI guidelines, KPC+MBL detection kit and Imipenem-EDTA method. Ten isolates were positive for MBL production and none of the isolates was KPC producing. The findings were in concordance with the study done by Datta et al who also performed MHT on 26 carbapenem resistant isolates of *Klebsiella* and none of the isolates was positive by MHT 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 cloxacinllin as inhibitor.\textsuperscript{[24]} Kumaraswamy et al reported 1.7\% isolates positive for KPC carbaenemases by MHT.\textsuperscript{[25]} Parveen et al reported 13.3\% of the isolates producing KPC enzymes by MHT.\textsuperscript{[20]} However higher rates were reported by other authors. Balan et al identified 33.3\% isolates positive by MHT.\textsuperscript{[26]} Rai et al identified 92 (90.2\%) isolates as carbapenemases producers by MHT.\textsuperscript{[27]} The present study showed 3.6\% prevalence of MBL enzymes in the isolates of \textit{Klebsiella} spp. by Imipenem-EDTA method. Slightly lower prevalence was reported by Deshmukh et al, who identified 1.25\% MBL producers using Imipenem EDTA method.\textsuperscript{[28]} Parveen et al performed Imipenem EDTA test on forty five meropenem resistant isolates and none of the isolate was found to be MBL producer.\textsuperscript{[20]} However, Pandya et al reported prevalence of MBL production in 7.26\% isolates of \textit{K. pneumoniae} which is higher than the present study.\textsuperscript{[29]} 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.

**CONCLUSION**

It is concluded that carbapenem resistant \textit{Klebsiella} play a crucial role in spreading UTIs and these pathogens are also extended from the hospital to community. Now, it is the need of the hour to improve infections control practices, avoid irrational use of antibiotics and empirical regime should be revisited to prevent further resistance. Researches on rapid detection of infectious microorganisms should be encouraged. We should also take immediate action to strengthen surveillance and laboratory capacity.

**REFERENCES**