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# Detection of Drug Resistant *Klebsiella Pneumoniae* from Various Clinical Samples in Tertiary Care Hospital, Central India: In Context with B-Lacatamase Enzymes (ESBL, Ampc, MBL and Carbapenemase)

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**Abstract** **Original Research Article**

Multi drug resistant (MDR) and extensive drug resistant (XDR) *Klebsiella pneumoniae* infection is very common and causes high morbidity and mortality in community acquired as well as hospital acquired infection. Here, we present a study to detect MDR, XDR and pan drug resistant and characterize extended spectrum β-lactamase, AmpC β-lactamase, metallo β-lactamase and carbapenemase producing *K. pneumoniae* isolates from different human clinical samples. A total 124 *K. pneumoniae* isolated from various clinical samples. Antimicrobial susceptibility of *K. pneumoniae* isolates was performed by Kirby-Bauer disk diffusion. The resistant isolates were tested for ESBL, AmpC, MBL and Carbapenemase production by their respective phenotypic confirmatory test. Distribution of MDR, XDR and PDR detected according to antimicrobial resistance pattern as per guideline. Total 124 *K.pneumoniae* isolated from various clinical samples, Isolates were maximum resistant to Ceftazidime 81% and least resistant to Imipenem 15%. 45% of *K. pneumoniae* was MDR, 30% were XDR and no isolate was PDR. ESBL production was seen in 48.3%, AmpC in 6%, MBL in 3.2% and Carbapenemase in 11% of isolates. The study indicates that inadvert uses of antibiotics promote the emergence, persistence, and dissemination of resistant isolates in the community as well as hospital environment. Periodic review of antibiotic policy is necessary for rationalized use of antibiotics.

**Keywords:** Multi Drug resistance, extensive drug resistance, Pan Drug resistance, *Klebsiella pneumoniae*, ESBL, Amp C, MBLand Carbapenemase.



## INTRODUCTION

Multidrug resistance is emerging worldwide at an alarming rate among a variety of bacterial species, including *K.pneumoniae.* Resistance determinants in *K.pneumoniae* are typically encoded on the chromosome, plasmids and many other mechanisms [1]. In 2013, the Centers for Disease Control and Prevention (CDC) released a landmark report on "Antibiotic Resistance Threats 2". Three microorganisms were tagged as posing a threat level of urgent – *Clostridium difficile*, carbapenem-resistant Enterobacteriaceae (CRE) and drug-resistant *Neisseria gonorrhoeae* [2]. CRE, which include organisms such as *Klebsiella pneumoniae* and *Escherichia coli*, are resistant to almost all currently available antibiotics [2]. Multi-drug resistance is also reported by the production of varying type of enzymes e.g. extended spectrum of β-lactamase, AmpC β-lactamase, metallo- β-lactamase and carbapenemase [3]. With the increase in occurrence and types of these multiple β-lactamase enzymes, early detection is crucial, the benefits of which include implementation of proper antibiotic therapy and infection control policy.

Extensive use of broad-spectrum antibiotics in hospitalized patients has led to increased carriage of Klebsiella and development of multidrug-resistant strains, extensive drug resistance and pan drug – resistant to various antimicrobial agents [4]. Pan drug-resistance implies non-susceptibility to all commercially available antibiotics relevant to the treatment of a particular bacterial infection. These strains are also isolated among nosocomial infections, particularly those in the intensive care unit (ICU).

Knowledge of antibiotic-susceptibility pattern of *K.pneumoniae* will be helpful so that hospital patients can be treated with more narrow- spectrum and target- specific antibiotics [5].

So, the present study was designed to investigate the presence of antimicrobial resistance of *K.pneumoniae* in our geographical area and the occurrence of different classes of β-lactamase enzymes in clinical isolates of *K.pneumoniae*.

## MATERIALS AND METHODS

A total of 124 consecutive, nonrepetitive isolates of *K. pneumoniae* isolated from different clinical samples like urine, blood, sputum, pus and body fluids ( CSF, Pleural fluid) etc. between Feb2015- July 2016. Samples were included in the study from Dept. of Microbiology, Ruxmaniben Deepchand Gardi Medical College (R.D.G.M.C.) and Chandrikabahan Ruxmaniben Gardi, Hospital (CRGH), Ujjain (M.P.). Samples were inoculated on appropriate culture media including blood agar and MacConkey agar as soon as received in laboratory and incubated for 18-24 hrs at 35-37$^0$C under aerobic condition, by using standard laboratory methods[3,6]. All the clinical isolates were identified by using standard guidelines [3, 7]. All isolates were stored at 4 ℃ in 0.2% semisolid agars until used. Antibiotic susceptibility testing was performed according to CLSI recommended Kirby-Bauer disk diffusion method [7]. The following antibiotics were tested for *K. pneumoniae*: Piperacillin(100 $\mu$g) ,Amoxicillin-clavulanate (20/10 µg), Piperacillin-tazobactam(100/10 µg), Amikacin(30µg), Gntamicin(10 $\mu$g), Tobramicin(10 $\mu$g), Cefuroxime (30µg), Cefepime (30µg), Cefoxitin (30$\mu$g), Cefotaxime (30$\mu$g), Ceftazidime (30µg), Cefuroxime (30$\mu$g),Ciprofloxacin(5µg), Norfloxacin (10 $\mu$g), Levofloxacin(5 $\mu$g), Nitrofurantoin (300 µg), Aztreonam (30 µg), Tetracycline (30µg), Co-trimoxazole (1.25/ 23.75 µg), Imipenem (10 µg), Meropenem (10 µg) and Ertapenem (10 µg). The zone diameters were interpreted as per CLSI recommendations [7]. *E.coli* ATCC 25922 strain was used for quality control.

Isolates that were resistant to third generation cephalosporins (3GC) were tested for ESBL production by combined disk diffusion method [7].

**Detection of extended spectrum β-lactamase (ESBL) Figure 1**

The screening for ESBL production was done as per recommended method [7]. Isolates showing zone of inhibition ≥ 22mm for Ceftazidime, ≥27 mm for Cefotaxime and ≥27 mm for Aztreonam were suspicious for ESBL production and isolates were tested by a phenotypic confirmatory test combined disc diffusion method. Discs of Ceftazidime (30µg) alone and Ceftazidime-clavulanic acid (30µg/10 µg) are placed 20 mm apart from centre to centre on the agar plate. An increase of ≥ 5mm in zone of inhibition with use of combination disc indicates the presence of ESBL [7]. *Klebsiella pneumoniae* ATCC 700603 serve as quality control.

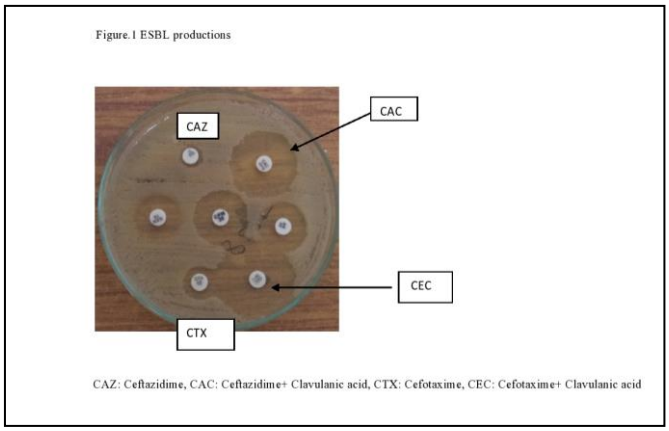


**Fig-1**

**Detection of AmpC β-lactamase (Fig 2)**

All the isolates that were screened for AmpC β-lactamase by Kirby–Bauer's disk diffusion method using cefoxitin (30 µg) disk. Zone of inhibition ≤ 18mm for cefoxitin was suspicious for AmpC production and is an indication for the organism to be tested by a phenotypic confirmatory test. [8,9,10]. Broth suspension of a cefoxitin susceptible *E.coli* ATCC 25922 indicator strain was adjusted to 0.5 McFarland's standard and plated on Muller Hinton agar plate by using of sterile cotton swab. After drying, cefoxitin (30µg) disc was placed at the centre of the plate and the test strains shown screening test positive streaked from the edge of the disc to the periphery of the plate. The plate was incubated overnight or 18-24 hours at 37$^0$C. The presence of a "diagonal" growth or 3mm or more in 'cloverleaf shaped' of zone of inhibition toward the test organism streak due to AmpC production by test strain was considered as positive. A negative Hodge test shows no diagonal growth into the cefoxitin zone [8, 9, 10].

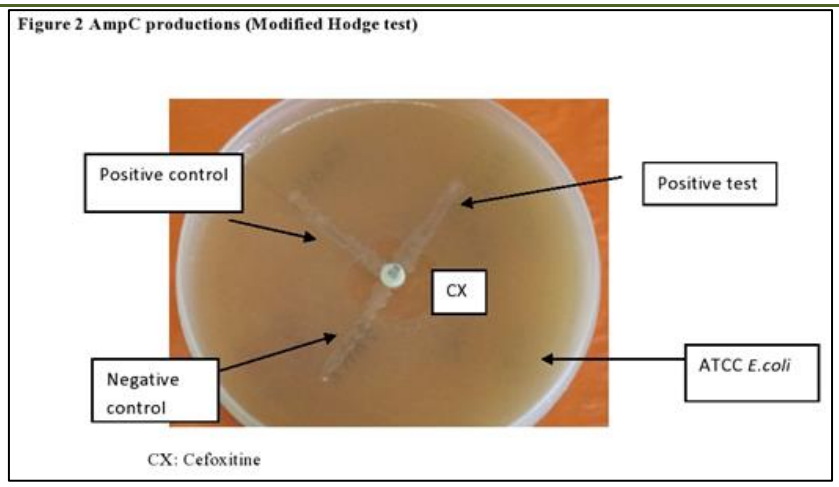


**Fig-2**

**Detection of Metallo-β-lactamase (Fig 3)**

Isolates that were resistant to carbapenemase (Imipenem, Ertapenam, Meropenam) and third generation cephalosporins (3GC) were considered screening positive. It is an indication for the organism to be tested by a phenotypic confirmatory test by Combined disc test (Zone enhancement with EDTA-imipenem disc) [7].Test organisms were inoculated onto plates of MHA. An Imipenem (10µg) disc and another Imipenem-EDTA disc were kept on the surface of the agar plate at the distance of 20 mm from centre to centre. The inhibition zones of Imipenem, and Imipenem-EDTA were compared after 16-18 hours of incubation in air at $35^0$C-37℃. Inhibition zone of Imipenem-EDTA disc is ≥ 7mm than the Imipenem disc alone, the strain is considered to be the MBL producer. [3, 7, 11].

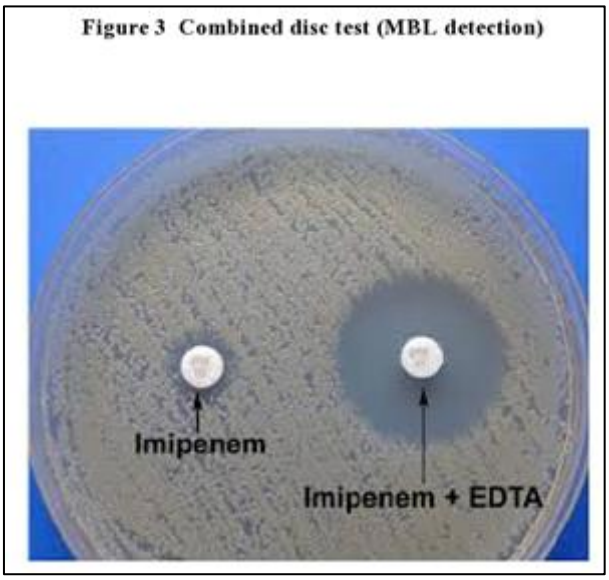


**Fig-3**

**Detection of Carbapenemase (Fig 4)**

Isolates were resistant to carbapenemase (Imipenem, Ertapenam, Meropenam) and third generation cephalosporins (3GC) were considered screening positive. The organism is tested by a phenotypic confirmatory test by Modified Hodge test. [7] Culture suspension of *E. coli* ATCC 25922 adjusted to 0.5 McFarland standards and diluted 1:10in saline or broth was inoculated using a sterile cotton swab on the surface of MHA. After drying for 3- 10 minutes, 10µg Imipenem disc was placed at the centre of the plate. Using of sterile loop, picked 3-5 colonies of the test strain was inoculated in a straight line out from the edge of the disc to the periphery of the plate. The streak was at least 20-25 mm in length. The plate was incubated at $37^0$C for 18-20 hours. The presence of a 'cloverleaf shaped' zone of inhibition due to carbapenemase production by test strain was considered as positive. *K. pneumoniae* ATCC BAA- 1705—MHT positive and *K. pneumoniae* ATCC BAA- 1706—MHT negative serve as controls [7].

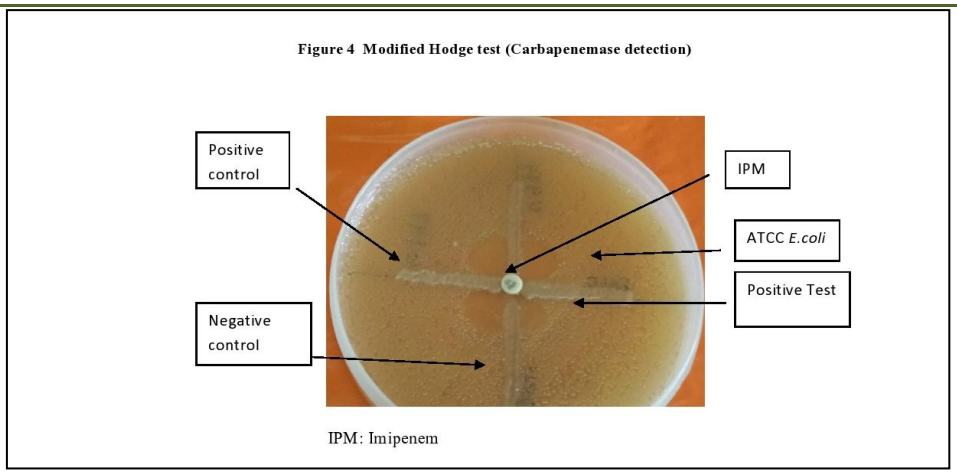


**Fig-4**

## RESULTS

A total of 124 *K.pneumoniae* were isolated from various clinical samples, among them male were 59% and female were 41%, as shown in Table 1.Thirty percent of *K.pneumoniae* were isolated from Surgery department followed by Pulmonary medicine 20% as shown in figure 5. Majority of *K.pneumoniae* isolates were in the age group of 0-10 years (18.5%) followed by 51-60 years of age group (17%) (Table 1). Isolates shown low level resistance to Imipenem 15.5%, Meropenem 27%, Ertapenem 27% with high level resistance pattern for Ceftazidime 81%, Cefotaxime 73.4 % and Amoxicillin-clavulanate 72.6 % (Figure 6). Urinary isolates were resistance to Norfloxacin 41% and Nitrofurantoin 37%. Distribution of MDR, XDR, PDR and ESBL, AmpC, MBL and carbapenemase, as shown in table 2 and 3 respectively.

**Table-1: Age and sex wise distribution of study subjects (n=124)**

| Age group(yrs) | No. of patients% | Male% | Female% |
|---|---|---|---|
| 0-10 | 23(18.5) | 15(12) | 8(6.4) |
| 11-20 | 9(7.2) | 4(3.2) | 5(4) |
| 21-30 | 19(15.3) | 2(1.6) | 17(14) |
| 31-40 | 19(15.3) | 10(8) | 9(7.2) |
| 41-50 | 16(13) | 11(8.9) | 5(4) |
| 51-60 | 21(17) | 17(14) | 4(3.2) |
| >60 | 17(14) | 13(10.4) | 4(3.2) |
| **Total** | **124 (100%)** | **73(59)** | **51(41)** |

**Table-2: MDR, XDR and PDR in *Klebsiella pneumoniae* isolates (n=124)**

| Antimicrobial category* | Number of Resistant | Percentages of Resistant |
|---|---|---|
| **MDR** | **56** | **45.2** |
| **XDR** | **37** | **30** |
| **PDR** | **00** | **00** |

*****β-lactams (Penicillin/Cephalosproin), carbapenems, Aminoglycosides, fluoroquinolones +Monobactams+ Cotrimoxazole +Tetracycline

MDR: [12] Non-susceptibile to≥1 agent in>3 antimicrobial categories
XDR: [12] Non susceptibile to ≥1 agent in all but <2 antimicrobial categories
PDR: [12]Non-susceptible to all antimicrobial agents

**Table-3: *Klebsiella pneumoniae* strains producing ESBL, AmpC, MBL and Carbapenemase enzymes (n=124)**

| Enzymes | Percentages(%) of Strains |
|---|---|
| **ESBL** | 48.3 |
| **AmpC** | 6 |
| **MBL** | 3.2 |
| **Carbapenemase** | 11 |

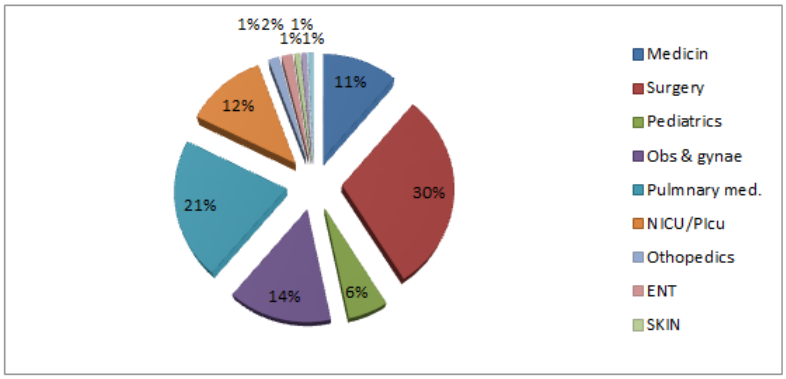


**Fig-5: Percentage of *Klebsiella pneumoniae* Isolates obtained from various departments (n=124)**

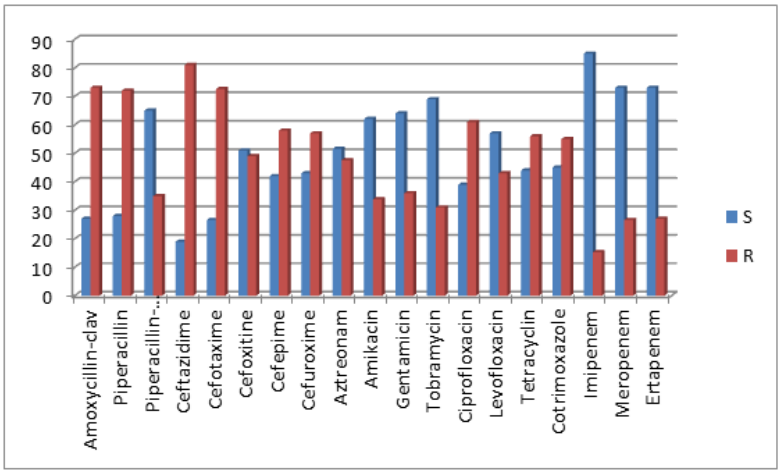


**Fig-6: Antibiogram of *Klebsiella pneumoniae In Percentages (n=124)***

## DISCUSSION AND CONCLUSION

In present study, majority of the strains were isolated from the patients of 0-10 year age group 18.5%, among them male were 12% and female were 6.4%. This is in contrast to a study conducted by Namratha KG *et al*. that reported highest strains from above 60 years group (male 28.57% & female 32.43%)[13]. In our study maximum isolates were obtained from Surgery 29.8%. Most of the strains were from the in-patients 88.7%, majority from males 60% compared to females 41.1%. This observation is in accordance with study from MGM hospital Mumbai (males 75% and females 53.05%) [14]. *K.pneumoniae* were isolated maximum from urine sample 40%, similar observation is reported from North India 41.5% [15]. But in work conducted from MGM hospital Mumbai, the highest rate of isolates were fom ET-secretion 40% [14]. *K. pneumoniae* from urine in our study might be due to the large number of urine samples received in the laboratory during the study period.

*K. pneumoniae* displays wide and variable spectrum of antibiotic resistance. In this study, high resistance was seen to Amoxicillin-clavulanic acid 72.6% and comparatively lower resistance to Aztreonam and Piperacillin-tazobatams 47.6% and 34.7% respectively. A study from Karnataka reported higher resistance to Aztreonam 83% and Piperacillin/tazobactum 67% [13]. In present study, resistance to third generation cephalosporin was 72.6%; with Ceftazidime and Cefotaxime showing 81% and 73.4% resistance respectively. It correlates with the study by Sasirekha *et al*. where 84% and 85% resistance to Ceftazidime and Cefotaxime respectively seen [16]. Another study by Archana Singh Sikarwar *et al*. reported variable range of resistance pattern to cephalosporins 28% to 76% [4]. This indicates that the frequent use of third generation cephalosporin and production of extended spectrum β- lactamase and Amp C β- lactamase may be accounting for widespread resistance. Although, resistance to aminoglycoside remains lower in our setup (Amikacin 33.9% and gentamicin 35.5% resistance). Much lower resistance was reported from Tamil Nadu (Amikacin 13.9, gentamicin19.3%) [17]. In our study, resistance to Ciprofloxaxin was 60.5%. This finding is in accordance with Study conducted by Gupta *et al*. 63% but studies by Rakesh Kumar and Ali *et al*. reported much higher percentages of resistance i.e. 88.8% and 76.9% respectively [18,19,20]. Looking into the resistance pattern, judicious use of antibiotic is advocated to circumvent the problems being faced by centres in larger cities as above.

Resistance to carbapenem was comparatively less in our study. Resistance to Imipenem was 15% whereas resistance to Ertapenem and Meropenem were 27%. Much less resistance to Meropenem 6.9% and Imipenem 4.3% was reported from Delhi study [21]. But a study conducted in tertiary care centre south India, reported higher resistance to Meropenem 43.6%, Imipenem 32% and Ertapenem 20.3% [22]. Study conducted by AIIMS showed a very high carbapenem resistance rate 69% [23]. In all studies, slightly more resistant to Meropenem might be due to its frequent use in the treatment of infections caused by multidrug resistant bacteria in ICU and high risk wards [24, 25].

Alarming increase in the emergence of MDR isolates among *K. pneumoniae* is a major problem. In our study, 45 % strains were MDR, 30% strains were XDR and PDR were not seen in any isolate. Our findings correlated well with study by Silpi Basak *et al.* they reported MDR 30% and XDR 27.8% [26]. In contrast, a study conducted from MGM hospital Mumbai, 67% strains were MDR [14]. A study conducted in Tertiary-Care Hospital in Beijing, China reported the proportion of MDR and XDR were 12.5%, 62.5% respectively, XDR strains were higher in Beijing than our study [27].

The increased incidence of drug resistant strains observed in our study may be because our hospital is a tertiary care center and patients from adjoining villages are admitted for treatment. Before attending the hospital, most of the patients get different antibiotics from general practitioners or due to over-the-counter sale of antibiotics often in improper dose.

The commonest mechanism of β-lactam antibiotic resistance in Gram negative bacteria is predominantly due to the production of β-lactamase that cleaves the structural β-lactam ring. In our study the ESBL production was 48.3%, higher number of ESBL production was detected from Surgery department 21%. Amp C production was 6% whereas MBL and Carbapenemase production was 3.2 % and 11% respectively. Maximum number of carbapenemase production was seen from NICU/PICU 6%. A study yielded ESBL 44.93% from Karnataka [28]. Another study by Singh *et al*. reported ESBL 35.32% and MBL 4.34% [29]. In a study from South India none of the isolates were found to produce MBL [22], Study by Anusuiya, AmpC was 5%, which correlates, with our study [30]. Contrast result reported by Varsha Gupta *et al*. for AmpC was 32% [18], which was higher than our study.

The emergence of drug resistant *K.pneumoniae* is a major threat to global health. MDR and XDR producing *K. pneumoniae* results higher morbidity and mortality and is due to various mechanisms. One of the most important drug resistance mechanisms is β-lactamase production; ESBL, MBL, Amp C and carbapenemase. B-lactamase can spread inside hospitals as well as outside in the community setting. Currently, few treatment options remain active against organisms that produce KPC and have resulted in the increased use of combination therapy. Until new effective drugs or combination of drugs are found, detection, prevention, and containment are the keys to curtailing the spread of this dangerous antimicrobial resistance.

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