

Original Research Article

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## Antibiotic Susceptibility Pattern and Extended Spectrum Beta Lactamase among *Klebsiella pneumoniae* isolates in a Tertiary Care Centre

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### ABSTRACT

#### Keywords

*Klebsiella pneumoniae*, Nosocomial Infections, Extended-Spectrum Beta Lactamases, Multidrug Resistance.

#### Article Info

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Extended spectrum beta-lactamase –*Klebsiella pneumoniae* (ESBL -KP) is one of the leading causes of nosocomial infections seen with increasing prevalence worldwide. The present study was undertaken to know the prevalence of ESBL-KP and their antibiotic susceptibility pattern and to create a baseline antibiotic resistance data to implement effective infection control policy. A total of 225 non duplicate *K. pneumoniae* isolates were obtained from various clinical samples over a period of 18 months. Antibiotic susceptibility testing and ESBL detection was done by Kirby Bauer's disc diffusion and CLSI methods respectively. Highest resistance of 91.5% was noted to amoxicillin-clavulanic acid. Least resistance was recorded to imipenem (24.4%) and amikacin (39.1%). Resistance of > 85% was noted to third generation cephalosporins [85.7% each to cefotaxime and ceftriaxone; ceftazidime (91%)]. ESBL positivity was 46.6%. ICU, wards and outpatient isolates revealed ESBLs of 51.3%, 39.7% and 52.7% respectively. Multi-drug resistance was 60%. Antibiotic resistance was higher in ICU isolates. Imipenem and amikacin may be preferred antibiotics in treating *K pneumoniae* infections.

### Introduction

*Klebsiellae* are opportunistic pathogens and can give rise to severe diseases such as septicemia, pneumonia, UTI and soft tissue infections. The hospitalized, immunocompromised patient with underlying diseases is the main target of these bacteria. The emergence and rapid spread of drug resistant *Klebsiella pneumoniae* isolates is becoming a serious antibiotic management problem

causing a great concern worldwide. (Jemima *et al.*, 2008)

*K pneumoniae* is considered the most medically important *Klebsiella* species, causing 75% to 86% of hospital acquired infections (Hansen *et al.*, 2004). Since the production of ESBL is frequently accompanied by broad-spectrum resistance, the ESBL positivity should be monitored

closely as the emergence of those highly drug-resistant *K. pneumoniae* strains will pose a serious impact on the remaining therapeutic options (Ben-David *et al.*, 2012). In a study based on the Tigecycline Evaluation and Surveillance Trial (TEST) global surveillance database, the rate of ESBL production was highest among the *K. pneumoniae* isolates collected in Latin America, followed by Asia / Pacific Rim, Europe, and North America (44.0%, 22.4%, 13.3% and 7.5%, respectively). Thus the potential of drug resistant *K. pneumoniae* to be a global health problem is great and more intensive surveillance of drug resistance in *K. pneumoniae* isolates is necessary in order to provide information for the development of effective diagnostic methods and drugs against the same. Hence the present study was performed to know the susceptibility pattern of *K pneumoniae* to various antibiotic groups and prevalence of ESBL strains in our hospital.

## Materials and Methods

The study was conducted between November 2012 to April 2014 at ESIC-MC and PGIMSR, Rajajinagar, Bengaluru, a tertiary care 500 bed teaching hospital. Clinical samples like pus, urine, sputum, blood, miscellaneous (throat swabs, vaginal swabs, body fluids) from both out-patients and inpatients submitted to diagnostic microbiology were included. *K pneumoniae* were isolated (only one isolate per patient included) and identified by standard methods. (Crichton, 2008)

## Antibiotic Susceptibility Testing [AST]

Antibiotic susceptibility test was performed by Kirby- Bauer's disc diffusion method using commercially available antibiotic discs [Hi Media, Mumbai, India]. Antibiotic disc included were ceftazidime [30µg], ceftazidime/clavulanicacid [30/10µg],

cefoxitin [30µg], cefotaxime [30 µg], cefoperazone [75µg], cefoperazone [75 µg]/sulbactam [10µg], ceftriaxone [30 µg], ciprofloxacin [5µg], amikacin [30 µg], amoxicillin/ clavulanicacid [20/10µg], aztreonam [30µg], cefepime [30µg], imipenem [10µg], piperacillin [100 µg], piperacillin/tazobactam [100/10µg], gentamicin [10µg], trimethoprim/ sulphamethoxazole [1.25/23.75µg].

A lawn of test pathogen prepared by evenly spreading 100 microliter inoculums with a sterilized swab onto Mueller Hinton agar plate. Antibiotic discs were gently and firmly placed on the agar plates, left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. If an antibiotic activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter at 24 hours using a measuring scale. An organism was interpreted as susceptible or resistant by comparing the zone of inhibition to standard chart.

## Screening for of ESBL

According to Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>[8]</sup>, strains showing zone of inhibition of  $\leq 22$  mm for ceftazidime,  $\leq 27$  mm for cefotaxime, and  $\leq 25$  mm for ceftriaxone in Kirby-Bauer's disc diffusion method were selected for confirmatory tests of ESBL.

## ESBL Confirmatory Test

### Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL<sup>[9]</sup>

ESBL production was confirmed among potential ESBL-producing isolates by phenotypic tests. Lawn culture of the organism was made and 3rd generation

cephalosporin- ceftazidime (30 µg) disc and ceftazidime + clavulanic acid (30 µg + 10 µg) disc was placed 25 mm apart [Fig.1]. An isolate showing increase of  $\geq 5$  mm in zone of inhibition for ceftazidime + clavulanic acid compared to ceftazidime alone was confirmed as ESBL producer.

### Statistical Analysis

Simple statistics to calculate percentage of antibiotic resistance in *Klebsiella pneumoniae* isolates were performed by entering the data into MicroSoft Excel and analysed. Statistical significance was assessed by calculating P value by Chi-square test.

### Results and Discussion

A total of 225 *K pneumoniae* were isolated during the study period. Fifty five (24.4%) were from outpatients, 72 (32%) were from the intensive care unit and 98 (43.5%) were from wards [Fig 2]. Eighty (35.6%) isolates were from females and 145 (64.4%) isolates were from males. Isolation from blood, pus, urine, sputum and miscellaneous was 18 (8%), 50 (22.2%), 53 (23.6%), 72 (32%) and 32 (14.2%) respectively [Fig 3]. Isolates of *K pneumoniae* revealed 46.6% of ESBLs. ICU, inpatients and outpatient isolates revealed ESBLs of 51.3%, 39.7% and 52.7% respectively.

Highest resistance of 91.5% was noted to amoxicillin-clavulanic acid. Least resistance was recorded to imipenem (24.4%) and amikacin (39.1%). Resistance of  $> 85\%$  was noted to third generation cephalosporins [85.7% each to cefotaxime and ceftriaxone; ceftazidime (91%)]. Resistance to gentamicin, piperacillin-tazobactam, cefoperazone-sulbactam, ciprofloxacin, co-trimoxazole and cefipime was 52.8%, 60.4%, 60.8%, 61.3%, 67.5% and 68% respectively (Table 1). Multi-drug resistance

was found to be 60%. Piperacillin-tazobactam, cefipime, gentamicin, amikacin and imipenem resistance among ESBL positive isolates were higher compared to ESBL negative isolates whereas cefoperazone-sulbactam, ciprofloxacin and co-trimoxazole resistance did not show significant (p value  $> 0.5$ ) difference among positive and negative isolates.

Antibiotic-resistant *K. pneumoniae* has been a noteworthy nosocomial pathogen for over 4 decades encountering resistance to third generation cephalosporins (3GC) through ESBLs.<sup>[10]</sup> In the present study, more than 85% isolates were resistant to 3GC and 68% percent were resistant to fourth generation cephalosporin (cefipime) and 46.6% were ESBL producers. Study from Punjab reveals 15.6% of *K pneumoniae* as ESBL producers whereas ESBL production was 46.2% in *K. pneumoniae* across Karnataka. The detection rates of ESBL-KP from different studies across Karnataka have been reported to vary from 9.6% (Bangalore) to 81.8% (Mangalore).

Recent study from Saudi Arabia shows highest percentage of ESBLs were from suction tip followed by blood, urine, and pus samples (Gupta *et al.*, 2007) and a similar reports have been presented by Gupta *et al* from India. (Babypadmini *et al.*, 2004) Other Indian studies from Coimbatore (Ananthan *et al.*, 2005) and Chandigarh on prevalence of ESBLs from urine and blood samples reports 40% and 69.2% respectively whereas sputum and urine isolates shows highest percentage of ESBLs in the present study. Prevalence of ESBL producers in any hospital depends upon various factors such detection methods used, antibiotic policy, the carriage rate among the hospital personnel, and the type of disinfectant used especially in the ICU.

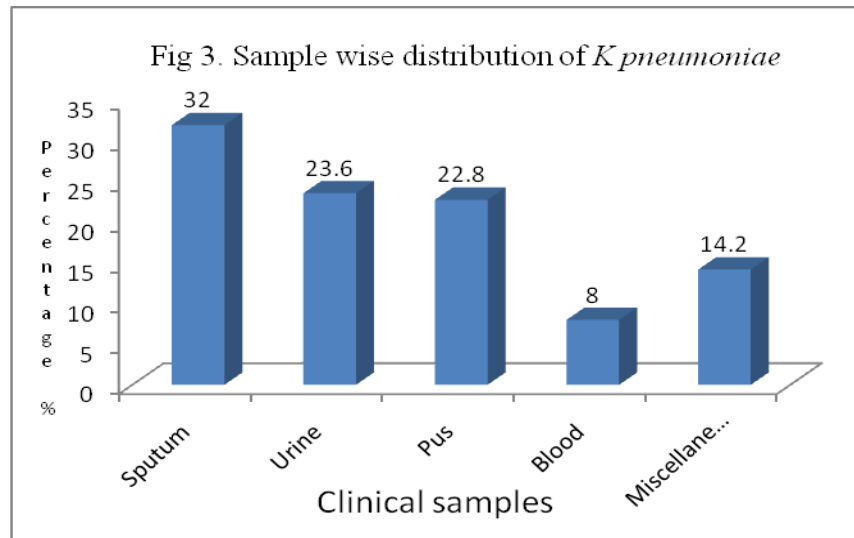
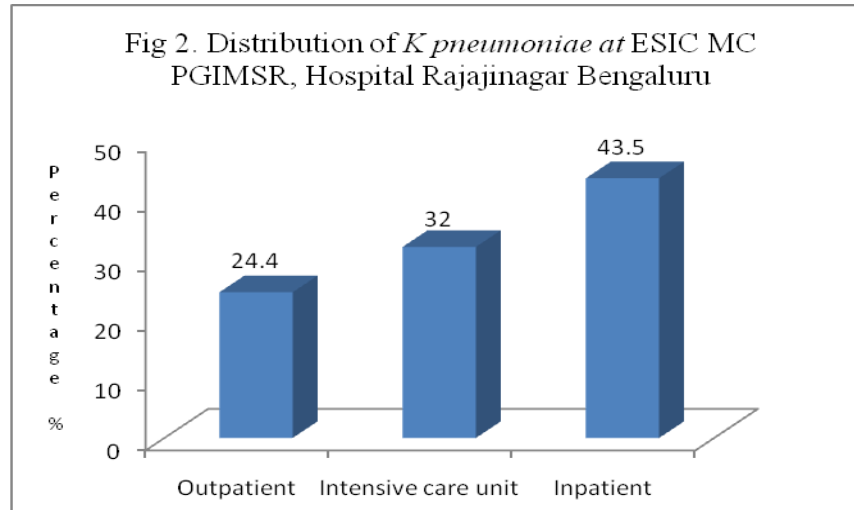
**Table.1** Distribution of Antibiotic Resistance (%) of *K pneumoniae* among different Patient Category

Patient category	Antibiotics									
	CTX	CAZ	CPZ	CPM	IM	AC	PTZ	AK	CF	COT
OP( 55)	45(81.8)	48(87.2)	44(80)	35(63.6)	8(14.5)	46(86.6)	28(50.9)	14(25.4)	26(47.2)	28(50.9)
IP (98)	83(84.6)	88(89.7)	83(84.6)	66(67.3)	23(23.4)	91(92.8)	63(64.2)	35(35.)	57(58.1)	63(64.2)
ICU(72)	65(90.2)	69(95.8)	65(90.2)	52(72.2)	24(33.3)	69(95.8)	45(62.5)	39(54.1)	55(76.3)	61(84.7)
Total (225)	193(85.7)	205(91.1)	192(85.3)	153(68)	55(24.4)	206(91.5)	136(60.4)	88(39.1)	138(61.3)	152(67.5)

OP:outpatient, IP: inpatient, ICU :intensive care unit, CTX: cefotaxime, CAZ: ceftazidime, CPZ: cefoperazone, CPM: ceftazidime, IM: imipenem, AC: amoxy-clavulanic acid, PTZ: piperacillin-tazobactam, AK: amikacin, CF: ciprofloxacin, COT: co-trimoxazole ( Numbers in parentheses represent percentage)

**Fig.1** ESBL Detection by Phenotypic Disc Diffusion Method





ESBL producing bacteria are typically associated with multidrug resistance and are most often encoded on plasmids, which can easily be transferred between isolates. (Podschun *et al.*, 1998) Multidrug resistance ESBL-KP is major cause of nosocomial infections. The present study shows a multidrug resistance of 60% with high resistance to all antibiotics except imipenem and amikacin which correlates with other studies where least resistance was recorded to carbapenems and amikacin.

In conclusion, the present study showed that the prevalence of ESBL-producing *K. pneumoniae* was high among in-patients. Imipenem and amikacin could be useful antibiotics against these resistant isolates. Detection of ESBL-KP by phenotypic confirmatory disc diffusion test is simple and cost effective, can be incorporated into routine laboratory practice to control the spread of these infections and allow a proper therapeutic strategy. Further study is needed to detect other resistance mechanisms in these isolates.

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