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Original Research Article.

Phenotypic Methods for Detection of Extended Spectrum β Lactamase Producing *Klebsiella pneumoniae* Isolated from Tertiary Care Centre, Bikaner

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ABSTRACT

Aim: The objective of study is to evaluate the prevalence of ESBL resistance in Klebsiella pneumoniae isolates and their antibiotic susceptibility pattern.

Method: All the samples received in the microbiology laboratory from the various departments were cultured on Blood and MacConkey's agar and blood was on Brain heart infusion broth. Antibiogram was done by Modified Kirby Bauer disk diffusion method. Detection of ESBL cases were done by double disc diffusion test and confirmed by E-test of CAZ/CAC.

Results: Total 1580 samples were tested in this study. Out of 1580, 207 Klebsiella pneumoniae isolates were found. Out of 207, 110 (53.14%) samples were found ESBL resistant klebsiella pneumoniae by double disc diffusion rest 97 (46.86%) was non-ESBL producer. Out of total 207 Klebsiella pneumoniae samples 63.28% were obtained from males and 36.72% from females. Maximum 98 (47.34%) cases were in between the age of 21-40 years. Out of total 207 cases of Klebsiella pneumoniae 112 ESBL resistant klebsiella pneumoniae by CAZ & CAC Ezy MIC™ Strips out of this, 64 Klebsiella pneumoniae were resistant with MIC ratio >256 mcg/ml.

Conclusion: ESBL resistant klebsiella pneumoniae poses serious problem choosing the right antibiotic for treatment. In order to prevent ESBL resistant klebsiella to emerge in a hospital/health care setup, various strategies such as strict infection control measures, judicious prescribing of antibiotics, antibiotics resistance surveillance programs and antibiotic cycling must be done. Regular monitoring and documentation of ESBL resistance has to be done, therefore it has been proposed that ESBL detection should be done in all microbiology laboratories.

Keywords: Extended Spectrum β Lactamase (ESBL), ESBL resistance, *Klebsiella pneumoniae.*

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INTRODUCTION

The rapid increase of antibiotic resistance threatens effective prevention and treatment of an also increasing range of infections. Klebsiella pneumoniae is among the bacteria which has showed high resistance in recent years and it can acquire resistance through mutations in some of their genes when they are exposed to an antibiotic. The main mechanism of antibiotic resistance mostly found is enzyme production such as β-lactamase enzymes. The β-lactamase enzymes produced by Klebsiella pneumoniae provide resistance to β-lactam antibiotics by hydrolyzing β-lactam rings.[1] The most widespread cause of resistance to beta lactam antibiotics is the production of enzymes called beta lactamases. Multidrug-resistant organisms (MDRO) "superbugs" by virtue of production of various β-lactamases confer resistance to many classes of antibiotics, particularly cephalosporins. The selective pressures which are generated by the indiscriminate use of the beta-lactam antibiotics have led to the selection of a variety of mutated forms of β-lactamases such as the ESBLs which has emerged as the most worrisome resistance mechanism posing a therapeutic challenge to the health care settings.[2]

ESBL are bacterial enzymes that hydrolyse and confer resistance to modern cephalosporin antibiotics. They constitute the major mechanism of resistance to second, third and fourth generation cephalosporins for example cefuroxime, cefotaxime, ceftriaxone and ceftazidime. Organisms often also possess resistance determinants to other antibiotic groups, such as aminoglycosides & fluoroquinolones leaving an extremely limited range of effective agents. SHV & TEM are the most common genes which are mainly responsible to cause ESBL production in K.pneumoniae [2]

This study was aim for the detection of Extended spectrum beta lactamase producing Klebsiella pneumoniae by phenotypic methods among the patients attending tertiary care centre, Bikaner.

MATERIALS AND METHODS

Samples were collected from various wards, Indoor & outdoor patient departments of PBM Hospital from Aug 2018 to Jan 2021. Clinical samples such as blood, CSF, urine, respiratory secretions, swabs from non-healing ulcers, pus/wound swab & other samples from sterile body fluids were collected by taking aseptic precautions. All the samples except blood were cultured on blood agar & Mac Conkey's agar. Blood culture was done on Brain heart infusion broth. A culture plates were incubated overnight at 37ºC. Isolated gram-negative organisms were further identified by standard set of biochemical tests.[3]

Klebsiella pneumoniae isolates from the various samples; which were having less sensitivity zone size of Ceftazidime on modified Kirby Bauer disk diffusion method were suspected and tested for ESBL detection by CAZ & CAZ Double disc diffusion test & by 'E strip-test'.[4]

Fig 1: K.pneumoniae on Mac Agar

Fig 2: Biochemical test of K. pneumoniae

1. Antibiotic Susceptibility Testing (Anti-biogram): Anti-microbial sensitivity testing was performed on Mueller Hinton agar (Hi-Media, Mumbai) plates by disk diffusion method according to CLSI guidelines[9]. The diameter of the zones of inhibition on MHA was interpreted as sensitive, intermediate and resistant. *Escherichia coli* ATCC 25922 (β-lactamase negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) strains were used as control organisms.

2. Double Disc Synergy Test (DDST) Using Imipenem and Imipenem Plus EDTA - Phenotypic method- double disc confirmatory test was performed on the Muller Hinton Agar (MHA) plates using sterile swabs. Ceftazidime (CAZ) disc of 30µg and Ceftazidime + Clavulanic acid (CAC) disc of 30+10µg concentration were placed at a distance of 30 mm centre to centre. The plates were incubated overnight at 37°C and readings were recorded. If difference in zone of inhibition of the antibiotic + inhibitor and antibiotic alone were ≥5mm, then the isolates were considered to be ESBL producers.[5] [Fig. 3]

Fig 3: ESBL *K. pneumoniae* (DDST)

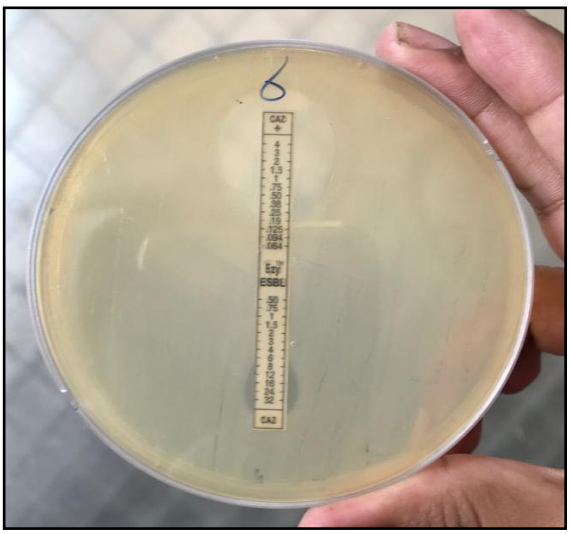

Fig 4: ESBL *K.pneumoniae* (E Test)

3. ESBL E-Test:

For MIC detection of Ceftazidime, the E-test strip method is used. The E-test ESBL strip (Hi-Media) containing a double sided even dilution range of Ceftazidime (CAZ) (MIC 0.25 to 16 µg /ml) gradient at one end & Ceftazidime+ Clavulanic acid (CAC) (0.001

to µg/ml) in incubation with a fixed concentration of Clavulanic acid at these other end. 100-mm-diameter Mueller-Hinton Agar plates are inoculated with swabs saturated with suspensions of the study isolates equivalent to a 0.5 McFarland standard. The results were read after 24 hrs of the incubation. The ratio of the MIC of CAZ/(CAC) > 8 dilution indicate MBL production[4]. [Fig 4]

RESULTS

Total 1580 non-repetitive variable samples from various wards were collected and processed in the Department of Microbiology, Sardar Patel Medical College and PBM Hospital, Bikaner.

Out of 1580, 207 (13.1%) Klebsiella pneumoniae were isolated. These 207 Klebsiella pneumoniae were further processed for the detection of ESBL resistance & other antibiotic sensitivity test by disk diffusion method on Muller Hinton Agar as per CLSI guideline.

Out of 207, 110 (53.14%) samples were found ESBL resistant klebsiella pneumoniae by double disc diffusion rest 97 (46.86%) was sensitive to carbapenems. Out of total 207 Klebsiella pneumoniae samples 63.28% were obtained from males and 36.72% from females. [Fig.5].

Maximum 98 (47.34%) cases were in between the age of 21-40 years. 90.82% Klebsiella pneumoniae were isolated from various wards & ICUs while only 9.18 % were found in OPD [Fig.6]. Out of 110 ESBL resistant Klebsiella pneumoniae cases, 32.85% were reported from Surgery ward infections while18% from wound infections, 13% of Urinary tract infections and 2% each from meningitis & bacteremia were found. [Fig. 7]

Various antibiotics included in the study were sourced from commercial batches belonging to β-lactam, aminoglycoside, quinolone, and tetracycline classes as per the CLSI guideline. Carbapenem resistant Klebsiella pneumoniae were not only resistant to Beta lactam group but also resist to most of antibiotics. Imipenem, Tigecycline and Colistin are only drug which showed more than 65% sensitivity in all ESBL resistant klebsiella pneumoniae isolates. [Table 1]

Out of total 207 cases of Klebsiella pneumoniae 112 ESBL resistant klebsiella pneumoniae by CAZ & CAC Ezy MIC™ Strips out of this, 64 Klebsiella pneumoniae were resistant with MIC ratio >256 mcg/ml. [Fig. 8]

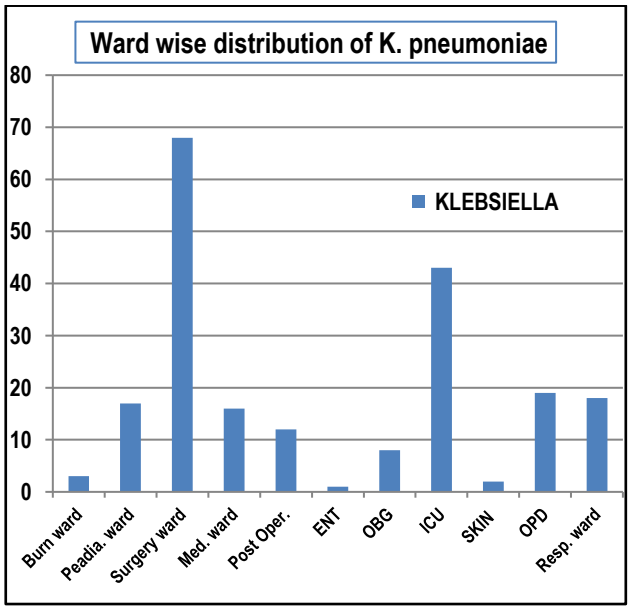

Fig 6: K.pneumoniae isolates from various wards.

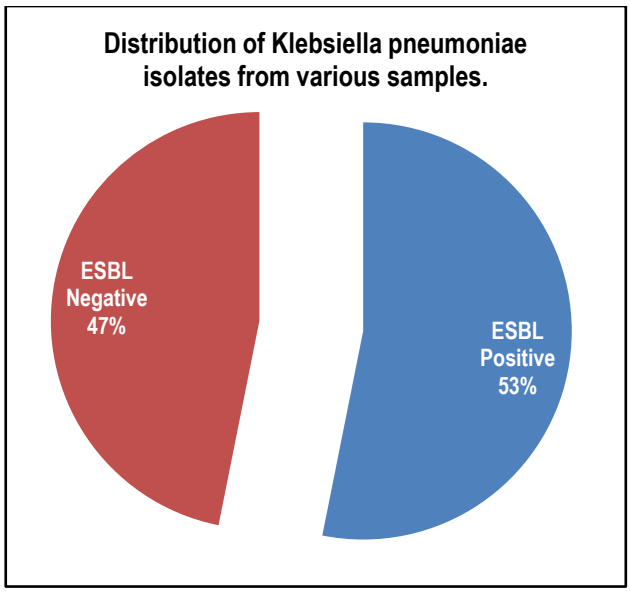

Fig 7: ESBL Positive & Negative K. pneumoniae isolates among present study.

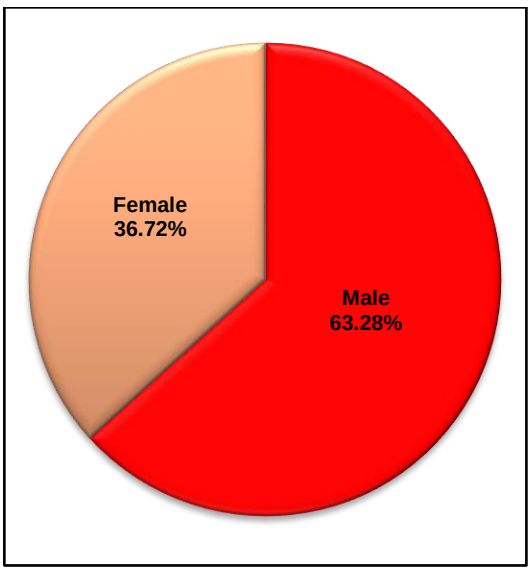

Fig 5: K.pneumoniae isolates from male & female

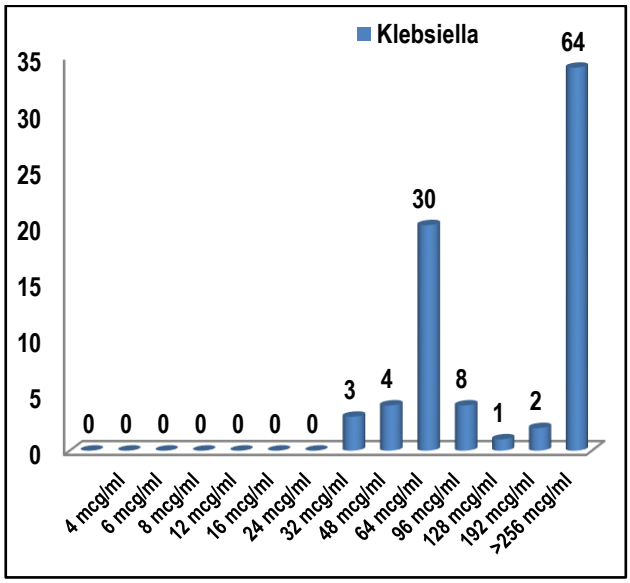

Fig 8: MIC level of Cefatazidime in Klebsiella pneumoniae

Table 1: Antibiotic sensitivity pattern of ESBL resistant Klebsiella pneumoniae clinical isolates

Antibiotic	Klebsiella pneumoniae (n=207)	%
Amikacin	83	40.0 %
Aztreonam	50	24.15 %
Ceftaxime	4	1.93 %
Cefoparazone-sulbactum	90	43.47 %
Ciprofloxacin	25	12.07 %
Colistin	201	97.10 %
Meropenem	94	45.41 %
Imipenem	134	64.73 %
Piperacillin- Tazobactum	68	32.85 %
Levo floxacin	47	22.7 %
Tigecycline	173	83.57 %

DISCUSSION

This study was conducted in the department of Microbiology, SPMC Medical College, Bikaner during the period of Aug, 2018 to Jan, 2021. The present study includes 207 clinically significant, consecutive, non-duplicate *Klebsiella isolates.* In the present study. Now a day's antibiotics have been used extensively and newer antibiotics are continuously being added for the treatment of various infections. An extensive use of β-lactam antibiotics in hospital and community has created a major problem leading to increased morbidity, mortality and health care costs.[6] Proper use of antibiotics is very important for various reasons. *Klebsiella* infections present a global medical challenge because it is an important opportunistic GNB in health care institutions. It has gained importance because of its ability to survive under a wide range of environmental conditions, having acquired drug resistance mechanisms and the emergence of multidrug and pan drug resistant strains.

In this study, the Multidrug resistance *Klebsiella* isolates were isolated predominantly from patients in Male surgery ward-49(23.67%) followed by ICUs including NICU & PICU 43 (20.77%), Female Surgery ward & OPD (9.17%), Male Respiratory ward (8.62%) and Peadiatric ward (8.21%) etc. This is similar to the study conducted by Vemula Sarojamma et al where most of isolates were obtained from ICU (19%) followed by surgery and trauma (13%).[7]

In the present study, ESBLs prevalence was 53.14%, which was very similar to the study done by 51.4% Shivaprakasha 2007; 53.4% Sasirekha 2010 60.98% Baby padmini 2004; & 58% Sheevani Sameer et al. Studies from India reported ESBL producers as high, 67.2% Guwahati 2014. Higher percentage (66.9%) was given by Gaurav Dalela et al, Bennett JW et al (64.8%), and Sharma et al. (2010) in India who found 70% ESBLs rate. while lower rates of ESBL were reported by Dechen C Tsering et al (34.03%), Meenu Garg et. al (41%), Vinothkumar et al (33.33%).[8-12]

The Antimicrobial sensitivity pattern of *Klebsiella* species were studied. In this study, the *Klebsiella pneumoniae* isolates were 97% Colistin, 83.57% Tigecycline 64% sensitive to Imipenem, Meropenem (45%), Piperacillin/tazobactum (32.85%), Amikacin (40%), Ciprofloxacin (12.07%). The results were interpreted as per CLSI guidelines. It correlates with the study done by Sasirekha et al. (2010) and Singh and Goyal (2003) in India.[13,14]

CONCLUSION

ESBL resistant klebsiella pneumoniae poses serious problem choosing the right antibiotic for treatment. In order to prevent ESBL resistant klebsiella to emerge in a hospital/health care setup, various strategies such as strict infection control measures, judicious prescribing of antibiotics, antibiotics resistance surveillance programs and antibiotic cycling must be done. Regular monitoring and documentation of ESBL resistance has to be done, therefore it has been proposed that ESBL detection should be done in all microbiology laboratories. Imipenem, Tigecycline & Colistin could be the drug of choice in ESBL resistant klebsiella infections. It should be used when no other drugs are effective.

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