

## PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF EXTENDED SPECTRUM B-LACTAMASE (ESBL) PRODUCING GRAM NEGATIVE BACILLI (KLEBSIELLA PNEUMONIA) IN A TERTIARY CARE TEACHING HOSPITAL, IN SOUTHERN-WEST KOTA DISTRICT OF RAJASTHAN (INDIA).

Gopal Lal Meena<sup>1</sup>, \*Ghanshyam Soni<sup>2</sup>, Deepak Maheshwari<sup>3</sup>

<sup>1</sup>Deputy Director, Department of Microbiology, District Hospital Rampura Attached Govt. Medical College, Kota, Rajasthan

<sup>2</sup>Senior Professor, Department of Microbiology, Govt. Medical College, Kota, Rajasthan

<sup>3</sup>Senior Demonstrator, Department of Microbiology, RNT Medical College, Kota, Rajasthan

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**Corresponding author:** Ghanshyam Soni

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### Abstract

**Background and Objectives:** The resistance to broad spectrum  $\beta$ -lactams which is mediated by the extended spectrum beta lactamase (ESBL) enzyme is an increasing problem now-a-days. This resistance mechanism has been responsible for nosocomial outbreaks, serious therapeutic failure if it is not detected on time and the outbreak of multidrug resistant, gram negative pathogens that need expensive control measures.

**Aim:** To know the prevalence of ESBL production in *klebsiella pneumoniae* and antibiotic sensitivity pattern to 3<sup>rd</sup> generation of cephalosporins in Govt. Medical College Kota.

**Material and Methods:** The study was conducted in Department of Microbiology, Kota Medical College, Kota, Rajasthan, from dec.2018 to dec.2019. Due permission from institutional ethical committee was obtained. This is descriptive observational study. All data were collected and analyzed with the help of suitable statistical parameters.

A total of 101 consecutive, nonrepetitive, gram negative isolates, which were resistant to one of the third generation cephalosporins (cefotaxime, ceftriaxone or ceftazidime) were selected as "Suspicious for ESBL production" as recommended by the Clinical and Laboratory Standards Institute (CLSI). These isolates were confirmed for ESBL production by the double disc synergy test (DDST) and the phenotypic confirmatory disc diffusion test (PCDDT) and they were further confirmed by the E-test ESBL strip randomly.

**Result:** Out of the 101 isolates by double disc synergy test (DDST) 45.54% (46) of *Klebsiella pneumoniae* isolates were ESBL producers, while PCDDT detected 58.41% (59) *Klebsiella pneumoniae* isolates as ESBL producers (p value < 0.05). Randomly selected isolates were further confirmed by the E-test ESBL strip, which showed a highly significant correlation with PCDDT.

**Key words:** Antimicrobial Susceptibility, *Klebsiella pneumoniae*, Gram Negative bacilli, Cephalosporins

### Introduction:

The application of antimicrobial chemotherapy in the treatment of infectious diseases, started in 20<sup>th</sup> century with introduction of Penicillin by Alexander Fleming in 1928, has considerably increases the life expectancy of human, domestic and wild animals. The emergence and spread of drug-resistant pathogens particularly multi- and pan-resistant bacteria (also known as "superbugs") are not treatable with existing antimicrobial medicines such as antibiotics.

Microbial resistance through Extended-Spectrum  $\beta$ -Lactamase (ESBL) was first reported in 1983 in Germany.<sup>(1)</sup> and subsequently in various parts of the world including India, soon after the introduction of third generation Cephalosporins in clinical practice. The production of  $\beta$ -

lactamase by gram-negative bacteria, usually *Klebsiella pneumoniae* and *Escherichia coli*, is the leading cause of resistance to the  $\beta$ -lactam antibiotics. The presence of multi-resistant plasmids with ESBL genes encoding extended-spectrum  $\beta$ -lactamases (ESBLs) that hydrolyze and cause resistance to oxyimino-Cephalosporins and aztreonam has been reported in virtually all species of Enterobacteriaceae, including *Klebsiella pneumoniae*, which greatly complicates the therapy for infections caused by them.<sup>(2)</sup> *Klebsiella pneumoniae* is a leading cause of hospital-acquired and various life threatening ailments such as urinary tract infections, septicemia, respiratory tract infections and diarrhea.<sup>(3,4, 5)</sup> Failure to detect ESBL-mediated resistance has led to treatment failure, an increase in hospital costs, length of stay, and patient mortality.<sup>(6)</sup>

The present study was planned to determine prevalence and antibiotic sensitivity pattern of ESBL producing *Klebsiella pneumoniae* isolated from nosocomial as well as community-acquired infections in the southern-west part (Kota) of Rajasthan (India).

### Materials and Methods

A descriptive study was conducted over a period of one year (**Dec. 2018 to Dec. 2019**) in Department of Microbiology, Kota Medical College, Kota, Rajasthan. The various clinical specimens such as pus, sputum, tracheal aspirate, cerebrospinal fluid, ascitic fluid, pleural fluid, blood and urine were collected from both the in-patients and the out-patients and received in our lab during the study period followed by isolation and identification of *Klebsiella pneumoniae* by conventional methods / standard bacteriological techniques. Patients' samples were inoculated on Blood agar, Mac Conkey agar and Nutrient agar and for sensitivity on Mueller-Hinton agar. The antibiotic sensitivity test performed by modified Kirby Bauer disc diffusion technique with commercially available Hi Media discs according to NCCLS guidelines on Mueller-Hinton agar plates. The isolated *Klebsiella pneumoniae* were tested for ESBL production by using Clinical Laboratory Standards Institute (CLSI) recommended guidelines for ESBL detection by both disc diffusion methods [Double Disc Synergy test (DDST).<sup>(7)</sup> and Phenotypic Confirmatory Disc Diffusion Test (PCDDT)<sup>(8)</sup> and MIC by E-test.<sup>(9,10)</sup>

This method is useful for both screening and Phenotypic confirmation of ESBL production.<sup>(11,12)</sup>

### Antibiotic Sensitivity Testing

The susceptibility of the ESBL producing bacteria to amikacin, gentamicin, ciprofloxacin, imipenem, Ceftazidime, Cefotaxime, and Ceftriaxone was determined by the Kirby-Bauer disk diffusion method according to the CLSI guide lines.

### Quality Control

*Klebsiella pneumoniae* ATCC 700603 (ESBL positive) was used as quality control for ESBL test. On disc diffusion testing the zone diameter ranges for *Klebsiella pneumoniae* ATCC 700603 are as follows:

Ceftazidime (10-18mm), Ceftriaxone (9-16mm), Cefotaxime (17-25mm). In PCDDT *Klebsiella pneumoniae* ATCC 700603 shows >5mm increase in ceftazidime/clavulanic acid zone diameter.<sup>(13)</sup>

**Ethics statement:** - The study protocol was approved by the institutional ethical committee. Informed consents were obtained in written form from patients and all clinical investigations were conducted according to the standard protocol. The patients gave consent for the publication of the clinical details.

**Data Analysis:** - Data was recorded as per Performa. The data analysis was computer based; SPSS-22 was used for

analysis. For categorical variables chi-square test was used. For continuous variables independent samples' t-test was used. p-value <0.05 was considered as significant.

### Results

**Table 1: Sample Distribution**

Sample	Number	%
Wound Swab	46	45.54%
Urine	37	36.63%
Sputum	14	13.86%
Vaginal Swab	02	1.98%
Throat Swab	01	0.99%
Pleural Fluid	01	0.99%
Total	101	100%

A total of 101 non-repeat isolates of *K. pneumoniae* were tested for ESBL production from various clinical samples, majority were from, wound swabs (46), urine (37), sputum(14), and vaginal swabs (2), throat swabs (1), Pleural fluid (1).

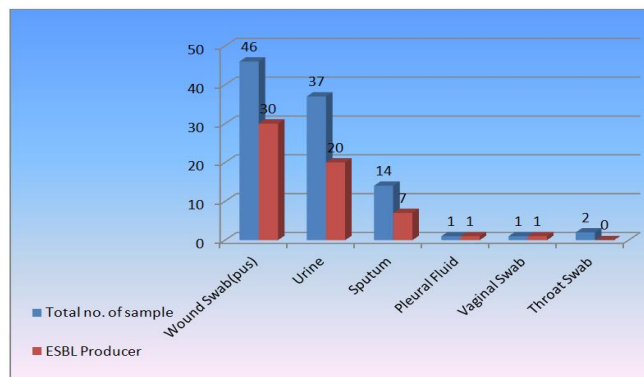
### Comparison of ESBL detection methods

#### ESBL Detection:

By double disc synergy test (DDST) 45.54% (46) of *Klebsiella pneumoniae* isolates were ESBL producers, while PCDDT detected 58.41% (59) *Klebsiella pneumoniae* isolates as ESBL producers (p value<0.05) [**Fig.-1**].



**Figure 1: Comparison of ESBL detection method**



**Figure 2: Sample wise ESBL Productions.**

The maximum ESBL Production was seen Wound Swab (50.8%), followed by Urine (33.8%), Sputum (11.8%) and others (p value <0.05).

#### Antimicrobial Susceptibility pattern:

The antibiotic sensitivity pattern revealed that the maximum sensitivity was seen for imipenem (100%), followed by gentamicin (53.46%), ceftriaxone (48.51%), amikacin (43.56%), cefotaxime (43.56%), ceftazidime (41.58%), ciprofloxacin (39.60%). High rate of resistance was seen for ciprofloxacin (60.39%), and for 3<sup>rd</sup> generation Cephalosporins; Ceftazidime (58.41%), Cefotaxime (56.43%), Ceftriaxone (51.48%).

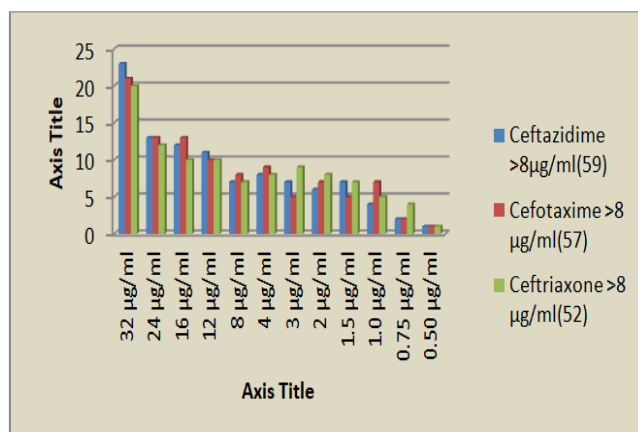
**Table 2: Antimicrobial Susceptibility pattern of all K. pneumoniae Isolates**

Antibiotic	Sensitive	%	Resistant	%
Amikacin	44	43.56%	57	56.43%
Gentamicin	54	53.46%	47	46.53%
Ciprofloxacin	40	39.60%	61	60.39%
Cefotaxime	44	43.56%	57	56.43%
Ceftazidime	42	41.58%	59	58.41%
Ceftriaxone	49	48.51%	52	51.48%
Imepenem	101	100%	00	0.00%

#### MIC determination:

59 (58.41%) of the K. pneumoniae isolates had MIC of >8µg/ml for ceftazidime, 57 (56.43%) for cefotaxime and 52 (51.48%) for ceftriaxone. Majority of the isolates among these had MIC >8µg/ml but MIC value detect all the isolates shows resistant or sensitive to the third generation of Cephalosporins (ceftazidime, cefotaxime, ceftriaxone) only confirmed ESBL Producer shows >8µg/ml value for ceftazidime (59), cefotaxime (57), ceftriaxone (52) (p value <0.05) [Fig.-3].

MIC of all ESBL positive K pneumoniae isolates were higher than the cut off point for each 3<sup>rd</sup> generation cephalosporin, i.e. >8µg/ml for ceftazidime, cefotaxime and ceftriaxone



**Figure 3: MIC ranges for 3<sup>rd</sup> generation Cephalosporins of all K. pneumoniae isolates**

**Table 3 : Third generation cephalosporin susceptibility pattern in ESB positive K pneumoniae isolates**

Antibiotic	Sensitive	%	Resistant	%
Ceftriaxone	49	48.51%	52	51.48%
Cefotaxime	44	43.56%	57	56.43%
Ceftazidime	42	41.58%	59	58.41%

The susceptibility of the ESBL producing K. pneumoniae to third generation Cephalosporins is depicted in Table-6. The resistance was highest to ceftazidime 59 (58.41%) followed by cefotaxime 57 (56.43%) and ceftriaxone 52 (51.48%).

#### Discussion

The existence of multiplicity of resistance mechanisms in Klebsiella pneumoniae isolates remain a grey area for most of the clinical Microbiologists. The emergence of plasmid encoded ESBLs in Klebsiella pneumoniae is because of extensive use of extended spectrum Cephalosporins since 1980s. The resistance to extended spectrum cephalosporins is mainly mediated by the production of ESBLs which is a significant evolution in antimicrobial resistance mechanism. (14)

In present study, out of 101 strains of Klebsiella pneumoniae 58.41% isolates were ESBL producers by Phenotypic confirmatory disc diffusion test. Klebsiella pneumoniae isolates with MIC >8µg/ml for ceftazidime, cefotaxime and ceftriaxone were identified as potential ESBL producers. In this study 58.41% of ESBL producing Klebsiella pneumoniae isolates had MICs >8µg/ml for ceftazidime.

By double disc diffusion test (DDST), 46 (45.54%) of Klebsiella pneumoniae were ESBL producers; while 59 (58.41%) were ESBL positive by Phenotypic confirmatory disc diffusion test (PCDDT) in this study. PCDDT method was found to be better method for ESBL detection which is also confirmed by E-test strip with 100% sensitivity, followed by DDST with 77.96% sensitivity.

**Table 4: Comparison of sensitivities of DDST and PCDDT**

Studies done	Sensitivity of DDST	Sensitivity of PCDDT
<b>Present study</b>	<b>77.96%</b>	<b>100%</b>
Amita Jain et al (15)	86.75%	93%
Priya Dutta et al (16)	40%	89%
Vikas Manchanda et al (17)	89%	100%

In this study ceftazidime was more effective in detecting ESBL producing Klebsiella pneumoniae with sensitivities of 58.41%, 77.96%, 100%, and 100% by routine disc diffusion test, DDST, PCDDT and E-Test respectively. It is established fact that ESBL producers show cross resistance to other antibiotics also, thus limiting the therapeutic choice. It was found in this study that multi-drug resistance was common in ESBL producing Klebsiella pneumoniae as compared to non-ESBL producers. Associated resistance

was found with ciprofloxacin (93%), gentamicin (88%), amikacin (84%), and netilmicin (79%). Khurana *et al.* <sup>(18)</sup> reported similar resistance pattern in ESBL producers to non  $\beta$ -lactam antibiotics with resistance maximum to amikacin (87%).

S Baby Padmini *et al.* <sup>(3)</sup> found high resistance to gentamicin and ciprofloxacin. All the ESBL positive *Klebsiella pneumoniae* isolates were sensitive to imipenem (100%).

Amita Jain and Rajesh Mondalet *et al.* <sup>(15)</sup> found that all ESBL positive isolats were sensitive to imipenem.

Shubha *et al.* <sup>(19)</sup> and Guillermo *et al.* <sup>(20)</sup> found similar results of imipenem sensitivity. Ekta Gupta *et al.* <sup>(21)</sup> found 6.9% of ESBL producers to be imipenem resistant, while Srujana Mohanty *et al* reported 5% imipenem resistance among ESBL producers.

In present study 59 (58.41%) of ESBL positive *Klebsiella pneumoniae* isolates showed resistance to any one of the third generation cephalosporins. Nosocomial infections due to ESBL producing present *Klebsiella pneumoniae* are of particular concern in intensive care units and other high risk areas like post-operative wards.

In present study high incidence of ESBL producing *Klebsiella pneumoniae* was found in in-patients (76.2%), specially from Burn Ward (33.89%), and Out-door (23.72%).

Mathur *et al* <sup>(2)</sup> reported 79% ESBL producers from ICUs and 50% from post-operative wards.

Bithika dutta Roy *et al.* (2005) <sup>(22)</sup> reported 58% ESBL producers from ICU. Another epidemiological study by Sadhegi *et al.* <sup>(23)</sup> found high prevalence of ESBL producing *Klebsiella pneumoniae* in in-patients (31.4%), as compared to 12.2% in out-patient group.

Despite the discovery of ESBLs, atleast two decades ago, clinical laboratories are still not fully aware of their importance. Confusion exists about the optimum test methods and appropriate reporting protocols. Failure to detect these ESBL producing microorganisms has contributed to their uncontrolled spread and the consequent clinical failures. As evident from our study *Klebsiella pneumoniae* is a potential nosocomial pathogen, with increased isolation from high pressure units such as Burn Ward, Medical ward and post-operative wards, and Out patients ,where newer  $\beta$ - lactam drugs are routinely prescribed. Phenotypic confirmatory disc diffusion test and MIC reduction (E-Test) test cannot be used as routine diagnostic procedures because of more time, cost effectiveness and labor involved in these procedures. In routine disc diffusion test ceftazidime can be used as representative drug to third generation Cephalosporins to test for antibiotic susceptibility. Ceftazidime was found to be having high MIC range and hence can detect most of the ESBL producing *Klebsiella pneumoniae*.

In routine disc diffusion technique Amoxycillin-clavulanate (30 $\mu$ g/10 $\mu$ g) disc can be placed near to ceftazidime disc 15mm apart from centre to centre. By this disc synergy can be done simultaneously along with other routinely used antibiotics for gram negative bacteria. By this ESBL producers can be detected simultaneously in the routine antibiotic susceptibility test.

Detection of ESBL producing *Klebsiella pneumoniae* by E-Test ESBL strip is sensitive and simpler method. As the strip carries two gradients; ceftazidime and ceftazidime with clavulanic acid, it can be used to determine MIC along with ESBL detection similar to Phenotypic confirmatory disc diffusion test.

The carbapenems like imipenem still retain adequate activity for almost all ESBL producing *Klebsiella pneumoniae*. We need to keep in mind that the carbapenems are antimicrobials that are usually kept in reserve. The marked increase in ESBL producers along with multi drug resistance, has left us with few alternative antimicrobials in combating serious infections.

This study emphasizes the continued surviellance of ESBL-producing *Klebsiella pneumoniae*. This will be helpful in monitoring antimicrobial resistance and to guide intervention to minimize its occurrence. Based on the prevalence of ESBL producers in a health care facility, antibiotic policy can be adopted by the institution. First line antibiotics should be choosen whenever these antibiotics have been reported as sensitive by clinical microbiology laboratory.

## Conclusion

We concluded that there is a need to formulate strategies to detect and prevent the emergence of ESBL producing *klebsiella pneumoniae* strains for the effective treatment of infections in in-door and out-door patients caused by them.

## ABBREVIATION:

**ESBL:** Extended spectrum  $\beta$ -lactamase

**CLSI:** Clinical and Laboratory Standards Institute

**MIC:** Minimum inhibitory concentration

**E Test:** Epsilometer test

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