

DETECTION AND ANTIBIOGRAM OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING KLEBSIELLA PNEUMONIAE IN SOUTH-EAST RAJASTHAN**Dr. Bhupendra Kumar Mandawat***

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ABSTRACT

Background: The spread of Extended Spectrum Beta-Lactamases (ESBLs) producing bacteria has become strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required. The therapeutic options for the infections which are caused by these organisms have also become increasingly limited. ESBLs producing strains have emerged as a significant challenge to counter with present antibiotics. **Objectives:** The aims of this study were to detect prevalence of ESBL production among *Klebsiella pneumoniae* and to detect their antibiotic susceptibility pattern at Govt. Medical College, Kota. This study demonstrate the importance of regular review of empirical antibiotic therapy in view of the evolving resistance of ESBL producing *Klebsiella pneumoniae* to commonly used antimicrobial agents. **Methodology:** A total of 101 consecutive, nonrepetitive *Klebsiella pneumoniae* isolates were studied. These isolates were confirmed for ESBL production by the double disc synergy test (DDST) and the phenotypic confirmatory disc diffusion test (PCDDT) and Twenty-five randomly selected isolates were further confirmed by the E-test ESBL strip randomly. Out of 101 *Klebsiella pneumoniae* isolate, 59(58.42%) were ESBL producers and 42(41.58%) were Non ESBL producers. Out of the 59 isolates which were tested, 42(71.19%) were found to be ESBL producers by PCDDT, 35(59.32%) were found to be ESBL producers by DDST and 18(72%), were found to be ESBL producers by the E-test ESBL strip which showed a highly significant correlation with PCDDT. **Conclusion:** As results showed that there was a high prevalence of ESBL production in our setup so, it is essential to report the ESBL production along with the routine sensitivity reports, which will help the clinician in selection of proper antibiotics.

KEYWORDS: ESBLs, KLEBSIELLA PNEUMONIAE, DDST, PCDDT, E-TEST.**INTRODUCTION**

Extended spectrum beta lactamases,^[1] (ESBL) are enzymes secreted by some Enterobacteriaceae most commonly *Escherichia coli* and *Klebsiella pneumoniae*.^[2,3] Extended spectrum β lactamases (ESBL) bacteria are emerging worldwide as a threat to favourable outcome in the treatment of common infections in community and hospital settings. They are mainly found in *Escherichia coli*, *Klebsiella* species and *Proteus* species but can also occur in other members of Enterobacteriaceae family.^[4] *Klebsiella pneumoniae* (*K. pneumoniae*) causes infections such as pneumonia, urinary tract infections, wound infection, cholecystitis and bacteriuria.^[1-4] In India, prevalence of ESBL producing *Klebsiella* spp. is reported varying from 6% to 87%.^[5] Extensive and often indiscriminate use of the extended spectrum cephalosporins in particular, Cefazidime, Cefotaxime and Ceftriaxone, is associated with the emergence and spread of multi drug resistant *K. pneumoniae*.^[6,25] Most of these organisms have developed resistance to antimicrobial agents. Inappropriate and empirical usage of wide spectrum antibiotics, insufficient

hygiene, immune suppression and prolonged hospitalization are some of the major aetiological factors that elevate the chances of infection.^[7, 25] Extended spectrum beta lactamases (ESBLs) producing bacteria are typically resistant to penicillins, first and second generation cephalosporins as well as the third generation oxyiminocephalosporins (e.g., Cefazidime, Ceftriaxone) and Monobactam (Aztreonam) except cephamycins and carbapenems.^[7,25] First isolated in 1983 in Germany, ESBLs spread rapidly to Europe, United States and Asia and are now found all over the world⁸. ESBLs detection is important because its spread within the hospital may lead to endemic occurrence and repeated outbreaks from time to time. Another important implication of ESBL production is failure to treat ESBL producing organisms because of limited therapeutic choices.^[9-10]

AIMS & OBJECTIVE

The aims of this study were to detect prevalence of ESBL production among *Klebsiella pneumoniae* isolates in various samples and to detect their antibiotic susceptibility pattern at Govt. Medical College, Kota.

This study demonstrate the importance of regular review of empirical antibiotic therapy for Klebsiella pneumoniae infections in view of the evolving resistance of ESBL producing Klebsiella pneumoniae to commonly used antimicrobial agents. This study is conducted to aid in early detection and treatment of ESBL producing Klebsiella pneumoniae and its prevention in community.

MATERIAL AND METHODS

The present prospective study was conducted in the clinical microbiology laboratory of the Govt. Medical College, Kota from period of 1st January, 2018 to 31th December 2018 to evaluate prevalence of ESBL production among Klebsiella pneumoniae isolates in various samples (different clinical specimens such as urine, pus, sputum etc) and to detect their antibiotic susceptibility pattern. A total of 101 consecutive, non-repetitive Klebsiella pneumoniae isolates were studied during this period. All samples were cultured on MacConkey Agar and Blood Agar and incubated at 37 °C for 24–48 hr. The isolates were identified and confirmed using standard microbiological methods including Gram staining, colonial morphology on media, growth on selective media, lactose and mannitol fermentation, H₂S production, catalase, oxidase, coagulase, indole and citrate utilization, and urease test. Antibiotic sensitivity testing of all Klebsiella pneumoniae isolates was performed on Muller Hinton agar (MHA) plates by Kirby-Bauer disk diffusion technique with guidelines established by the Clinical Laboratory Standards Institute⁶ (CLSI). All Klebsiella pneumoniae isolates were included in the study. Antibiotic Susceptibility testing to various antimicrobial agents was determined by Disc diffusion method of Kirby Bauer on MHA (Hi-media) as described by the Clinical Laboratory and Standard Institute (CLSI) guidelines.^[6] The following antibiotic discs (drug concentration in µg) were used: Ampicillin (10 µg), Amoxicillin-clavulanic acid (20/10 µg), Piperacillin (100 µg), Piperacillin-tazobactam (100/10 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5µg), Co-trimoxazole (1.25/23.75 µg), and Imipenem (10 µg). Phenotypic evidence of ESBL production was tested by the combination disk method MIC reduction test as per guidelines of CLSI.^[6]

Test for ESBL Production

A. Screening Test-ESBL detection was done for all isolates according to latest CLSI criteria. All Klebsiella pneumoniae isolates were subjected to screening tests by using Cefotaxime (30 µg) and Ceftriaxone (30 µg) discs. Those isolates with Cefotaxime zone ≤27 mm and Ceftriaxone zone ≤25 mm were considered as ESBL producer and then those isolates were subjected to confirmatory tests.^[6]

B. Confirmatory Test

1. Double Disc Synergy Test (DDST): According to the British Society for Antimicrobial Chemotherapy (BSAC)

guidelines isolates which were presumed to be ESBL producers on the basis of the screening test results, were picked up and emulsified in saline to a 0.5 McFarland's turbidity standard. Discs of Ceftazidime (30 µg), Cefotaxime (30 µg) and Amoxycyclav (20 µg Amoxycillin and 10 µg Clavulanic acid) were placed at a distance of 20 mm from center to center in a straight line, with the Amoxycyclav disc in the middle on a plate of MHA being inoculated with the test strain. The plates were incubated at 37 °C aerobically overnight. Isolates which showed an enhancement of the zone of inhibition as greater than 5 mm on the Amoxycyclav side of the disc as compared to that which was seen on the side without Amoxycyclav, were confirmed as ESBL producers.^[6] (Figure 1).



Figure 1: Double disc Synergy Test (DDST)-Organism showing enhanced zone of inhibition between ceftazidime and cefotaxime and amoxicillin/clavulanic acid containing disc indicating ESBL production.

2. CLSI Confirmatory Test (PCDDT-Phenotypic Confirmatory Double Disc Test)

For this test disc of Ceftazidime (30 µg) and Ceftazidime plus Clavulanic acid (30/10 µg) were placed on MHA and incubated. An increase of > 5 mm in the zone of inhibition of the combination discs in comparison to the Ceftazidime disc alone was considered to be a marker for ESBL production. E.coli ATCC 25922 and K. pneumoniae ATCC 700603 were used as negative and positive controls, respectively.^[6] (Figure 2).



Figure 2: PCDDT (Phenotypic Confirmatory Double Disc Diffusion Test)-A > 5 mm increase in zone of inhibition for ceftazidime/clavulanic acid (CAC) versus its zone diameter when tested alone by ceftazidime confirmed an ESBL producing organism.

3. The ESBL E Test: The E-test ESBL strips (AB Biodisk, Sweden).^[11] carry two gradients, ceftazidime (0.5-32 µg/ml) on the one end and ceftazidime plus clavulanic acid (0.064-4 µg/ml) in a different concentration gradient on the other end, along with a fixed concentration of clavulanic acid (4 µg/ml). A lawn culture of the test organism was plated on Mueller Hinton Agar (MHA) on which the E-test ESBL strip was placed on the centre of the plate. The plates were incubated aerobically at 37°C for 16-18 hours. The MIC was interpreted at the point of intersection of the inhibition eclipse with the E-test strip edge. The presence of ESBL was confirmed by the appearance of a phantom zone or by the deformation of the TZ eclipse or when the ceftazidime MIC was reduced by >3 log₂ dilutions (ratio TZ/TZL, >8) in the presence of clavulanic acid [Fig-3] as per the manufacturer’s guidelines.^[11] (Figure 3).



Figure 3: Epsilon test(E-test):-E-test ESBL strip showing clear cut ESBL positive organism showing ceftazidime (TZ) MIC is reduced by >3 log₂ dilutions (ratio TZ/ TZL, >8) in the presence of clavulanic acid.

Quality control: β-lactamase negative Escherichia coli ATCC 25922 was used as the negative control and ESBL-producing Klebsiella pneumoniae ATCC 700603 was used as the positive control throughout the study.^[6]

RESULT

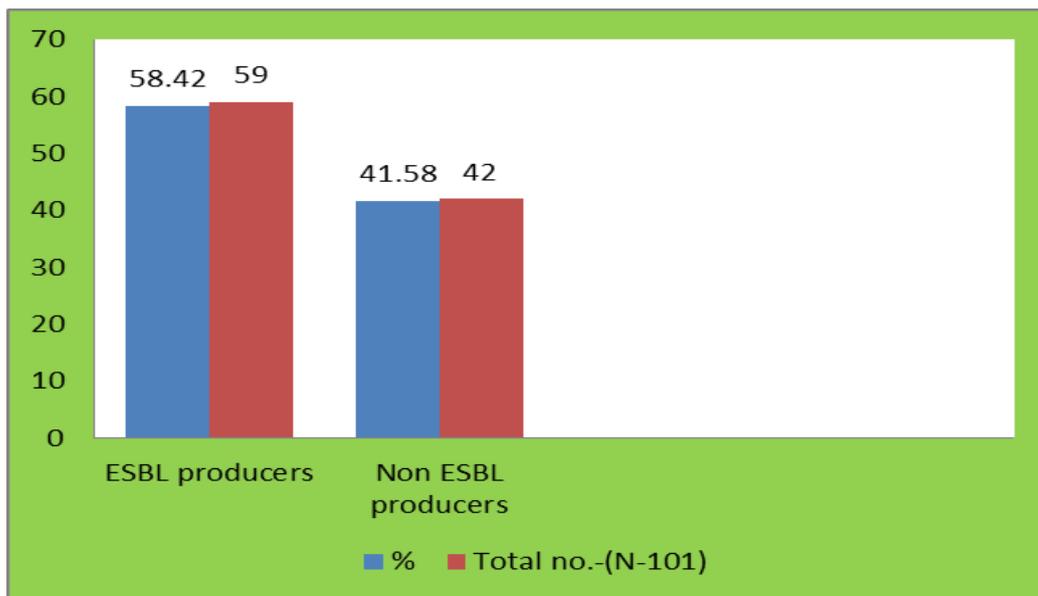
A total of 101 consecutive, nonrepetitive Klebsiella pneumoniae isolates were studied. Antimicrobial susceptibility test was performed on Muller Hinton agar plates using Kirby Bauer disk diffusion method. ESBL detection was done for all isolates according to latest CLSI criteria. These isolates were 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. Twenty-five randomly selected isolates were confirmed by the E-test ESBL strip. Out of 101 Klebsiella pneumoniae isolate, 59(58.42%) were ESBL producers and 42(41.58%) were Non ESBL producers.

Table 1: Age wise and Sex wise distribution of ESBL positive Klebsiella pneumoniae isolates.

Age group (years)	Male	Female	Total no. of cases (%) (n=101)
0-15	3	2	05 (4.95%)
15-30	13	10	23 (22.77%)
30-45	10	6	16 (15.84%)
45-60	27	22	49 (48.52%)
>60	5	3	08 (7.92%)
Total cases	58 (57.43%)	43 (42.57%)	101

Table 2: ESBL producers among Klebsiella pneumoniae isolates.

ESBL producers among Klebsiella pneumoniae isolates	ESBL producers		Non ESBL producers	
	Number	%	Number	%
101	59	58.42 %	42	41.58 %



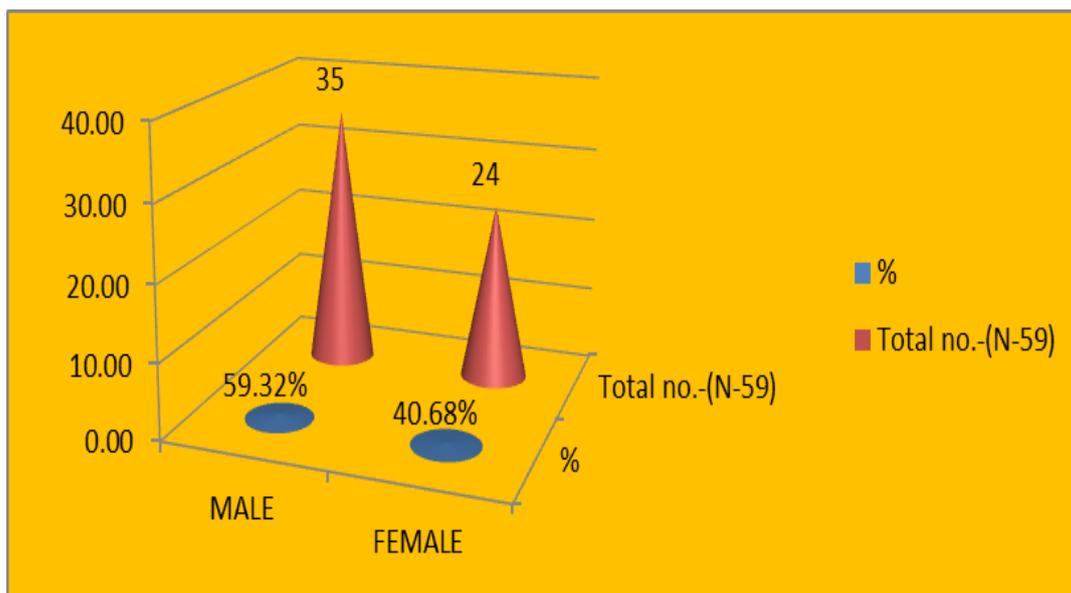
Graph 1: ESBL producers among Klebsiella pneumoniae isolates

Table 3: Distribution of ESBL and non-ESBL producers Klebsiella pneumoniae in various samples.

Various Samples	ESBL producers (n=59)	Non ESBL producer(n=42)	Total (%) (n=101)
Urine	20 (33.90%)	17 (%)	37 (36.63 %)
Sputum	07 (11.86%)	07 (%)	14 (13.86%)
Throat swab	00 (0%)	02 (%)	02 (1.98%)
Wound swab	30 (50.88%)	16 (%)	46 (45.54 %)
Blood	01 (1.69%)	00 (%)	01 (0.99%)
CSF	01 (1.69%)	00 (%)	01 (0.99%)
Total cases	59 (58.42 %)	42 (41.58 %)	101

Table 4: Sex wise distribution of ESBL producers Klebsiella pneumoniae

Total number of ESBL isolates	ESBL producers in males		ESBL producers in females	
	Number	%	Number	%
59	35	59.32%	24	40.68%



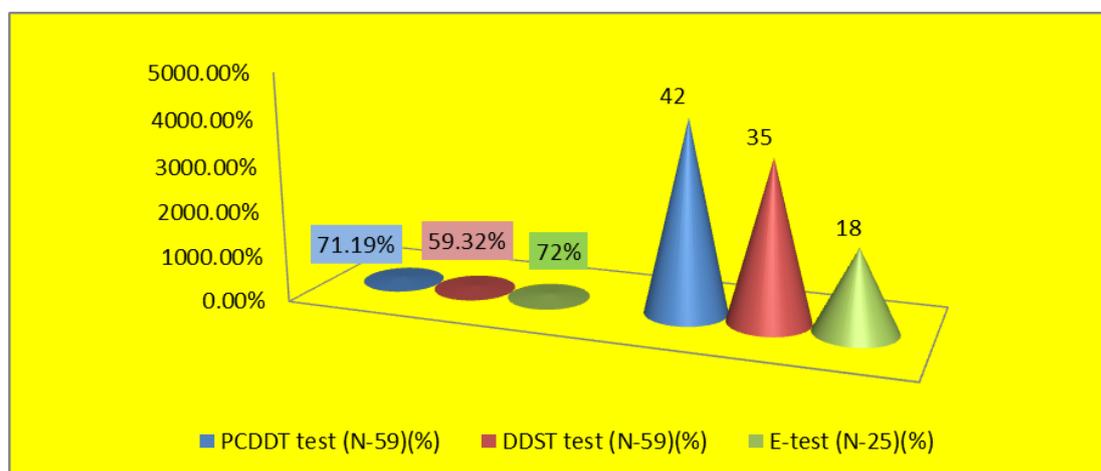
Graph 2: Sex wise distribution of ESBL producers Klebsiella pneumoniae.

Table 5: ESBL producers *Klebsiella pneumoniae* distribution in ward-wise with sex wise.

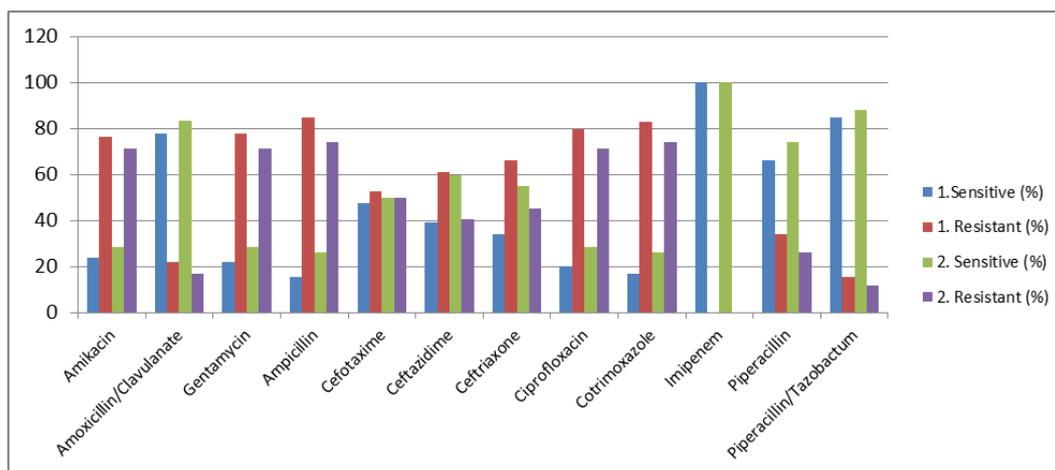
S. No.	Name of wards	Total Samples	Male	Female	ESBL Producer			Total (%) (n-59)
					Male	Female	Total	
1	Surgical ward	13	09	04	04	01	05	05 (8.47 %)
2	Orthopaedic ward	08	05	03	04	02	06	06 (10.16%)
3	Burn ward	28	16	12	11	09	20	20 (33.89%)
4	Post Operative ward	02	00	02	00	01	01	01 (1.69%)
4	OPD (Out-door)	23	13	10	08	06	14	14 (23.72%)
5	Medical ward	17	10	07	05	03	08	08 (13.55%)
6	ICU	04	03	01	02	01	03	03 (5.08%)
7	Cottage ward	03	02	01	01	01	02	02 (3.38%)
8	Stroke ward	01	00	01	00	00	00	0 (0%)
9	Obstetrics-gynaecology ward	01	00	01	00	00	00	0 (0%)
10	Paediatric ward	01	00	01	00	00	00	0 (0%)
	Total no. of cases	101	58	43	35	24	59	59 (100%)

Table 6: ESBL Producing *Klebsiella pneumoniae* by Various Confirmatory tests.

Organism (N-59)	PCDDT test (N-59) (%)	DDST test (N-59) (%)	E-test (N-25) (%)
<i>Klebsiella pneumoniae</i> (59)	42(71.19%)	35(59.32%)	18 (72%)

**Graph 3: ESBL Producing *Klebsiella pneumoniae* by Various Confirmatory tests****Table 7: Antibiotic susceptibility pattern of ESBL producing *Klebsiella pneumoniae* isolates.**

Antibiotics (N-101)	ESBL producer (N-59)		Non ESBL producer (N-42)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Amikacin	14 (23.73%)	45(76.27%)	12(28.57%)	30(71.43%)
Amoxicillin/Clavulanate	46 (77.97%)	13(22.03%)	35(83.33%)	07(16.67%)
Gentamycin	13 (22.03%)	46(77.97%)	12(28.57%)	30(71.43%)
Ampicillin	09 (15.25%)	50(84.75%)	11(26.2%)	31(73.8%)
Cefotaxime	28 (47.46%)	31(52.54%)	21(50%)	21(50%)
Ceftazidime	23 (38.98%)	36(61.02%)	25(59.52%)	17(40.48%)
Ceftriaxone	20 (33.90%)	39(66.10%)	23(54.76%)	19(45.24%)
Ciprofloxacin	12 (20.33%)	47(79.66%)	12(28.57%)	30(71.43%)
Cotrimoxazole	10 (16.95%)	49(83.05%)	11(26.2%)	31(73.8%)
Imipenem	59 (100%)	0(0%)	59(100%)	0(0%)
Piperacillin	39 (66.10%)	20(33.90 %)	31(73.8%)	11(26.2%)
Piperacillin/Tazobactam	50 (84.75%)	09(15.25%)	37(88.10%)	05(11.90%)



Graph 4: Comparative study of Antibiotic susceptibility pattern in both ESBL producers and non ESBL producers in percentage (%) (Total no. -(N-101) 1.ESBL producer(N-59) 2. Non ESBL producer(N-42).

Table1 shows age wise and sex wise distribution of ESBL producing *Klebsiella pneumoniae* isolates. Out of 101 *Klebsiella pneumoniae* isolate, 58(57.43%) were found in males and 43(42.57%) in females and most commonly age groups were affected 45-60yrs (48.52%), 15-30yrs(22.77%),30-45yrs(15.84%), >60yrs (7.92%) and 0-15yrs(4.95%) in decreasing order. Table2 and Graph1 show the number and percentage of ESBL and Non ESBL producing *Klebsiella pneumoniae* isolates. Out of 101 *Klebsiella pneumoniae* isolate, 59(58.42%) are ESBL producers and 42(41.58%) are Non ESBL producers. Table3 shows distribution of ESBL and non-ESBL producers *Klebsiella pneumoniae* in samples. *Klebsiella pneumoniae* was isolated mainly from wound swab (45.54%), urine (36.63%) and sputum (13.86%) samples. Table4 and Graph2 shows the number and percentage of ESBL producing *Klebsiella pneumoniae* in males and females. Out of 59 ESBL producing *Klebsiella pneumoniae*, 35(59.32%) were found in males and 24(40.68%) in females. Table5 shows ESBL producers *Klebsiella pneumoniae* distribution in ward-wise with sex wise. ESBL producers *Klebsiella pneumoniae* was isolated mainly from Burn ward (33.81%), OPD (23.72%) and Medical ward (13.85%). Table6 and Graph3 show the ESBL Producing *Klebsiella pneumoniae* by Various Confirmatory tests. Out of 59 ESBL producing *Klebsiella pneumoniae*, 42(71.19%) were found to be ESBL producers by PCDDT and 35 (59.32%) were found to be ESBL producers by DDST and out of 25 samples which were further confirmed by the E-test ESBL strip,18(72%) were found to be ESBL producers. Table7 and Graph4 shows the antibiotic susceptibility pattern of ESBL producing *Klebsiella pneumoniae* isolates. The isolates were highly susceptible to Imipenem (100%) and Piperacillin/Tazobactam (84.75%) and Amoxycylav (77.97%) in the decreasing order, were the most active and reliable agents for the treatment of the infections which were caused by the ESBL producing organisms and were resistant to Ampicillin (84.75%), Cotrimoxazole

(83.05%) and Ciprofloxacin (79.66%) drugs. There is a scarcity of information on ESBL prevalence particularly in developing countries like India, hence present study was conducted for early detection & prevention of ESBL producer organisms.

DISCUSSION

The present study was conducted in the Department of Microbiology & Immunology, Govt. Medical College, Kota. The patients included in the study were of OPD and IPD from all associated hospitals of Govt. Medical College, Kota reporting for diagnosis in Department of Microbiology from 1st January, 2018 to 31th December 2018 to evaluate prevalence of ESBL production among *Klebsiella pneumoniae* isolates and to detect their antibiotic susceptibility pattern. The samples were collected and processed as per routine recommended methods of technical guidelines. In all, 101 patients were screened. The observations were made with reference to age, sex, constitutional symptoms, various risk groups and investigations. ESBL prevalence varies with geographical distribution and social characteristic of population groups. Prevalence of ESBL varies across continents, countries and hospitals as demonstrated by various studies. Correct identification of ESBL producing organisms in due time is necessary not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organism. Our study was a step ahead in this direction with the purpose of providing authentic scientific data based on the affected population attending our hospitals. ESBL positive *Klebsiella pneumoniae* which indicates great variation in ESBL positivity throughout India and world. (Table 8 and Table 9).

Table 8: Various studies showing the prevalence of ESBL producing Klebsiella pneumonia.

S. No	Studies	Place	Year	Prevalence (%)	S. No	Studies	Place	Year	Prevalence (%)
1.	Purva Mathur et al. ^[21]	New Delhi	2002	68%	8.	Goyal et al. ^[17]	Lucknow	2009	63.6%
2.	C Rodrigues et al. ^[9]	Mumbai	2004	53%	9.	Mahesh E et al. ^[19]	Bengaluru	2010	56.2%
3.	S.Babypadmini et al. ^[13]	Coimbatore	2004	40.3%	10.	Manoharan et al. ^[20]	Vellore	2011	78%
4.	S Singhal et al. ^[23]	Gurgaon	2005	64%	11.	Dissanayake et al. ^[15]	Srilanka	2012	29%
5.	Kumar et al. ^[18]	Hyderabad	2006	24.8%	12.	Chaudhary NK et al. ^[14]	Mysore	2013	54.5%
6.	Hawser SP et al. ^[8]	Asia Pacific Region	2007	79%	13.	Dugal S et al. ^[16]	Mumbai	2013	24.4%
7.	Sridhar Rao et al. ^[24]	Karnataka	2008	62.9%	14.	Present Study	Kota	2018	58.42%

Table 9: Various studies showing Comparison of sensitivities of DDST and PCDDT.

Studies	Sensitivity of DDST	Sensitivity of PCDDT
Priya Dutta et al. ^[22]	40 (%)	89 (%)
Vikas Manchanda et al. ^[5]	89 (%)	100 (%)
Amita Jain et al. ^[12]	86.75 (%)	93 (%)
Present Study	59.32 (%)	71.19 (%)

Our findings were in accordance with various authors as above. Comparison of studies conducted by other researchers showed slight variations in prevalence of ESBL prevalence. This study agrees with previous studies in other countries as well as India. Previous studies from India have reported the prevalence of the ESBL producers to be 6 to 87%.^[5] In our study the ESBL prevalence was 58.42%. The our results were correlated with the Previous studies in India by Purva Mathur et al.^[21] S Singhal et al.^[23] Sridhar Rao et al.^[24] Goyal *et al.*^[17] Mahesh E et al.^[19] and Chaudhary NK et al.^[14] in which ESBL prevalence were 68%,64%,62.9%, 63.6%, 56.2% and 54.5% respectively whereas in our study it was 58.42%. The our results were not correlated with the Previous studies in other countries studies by Hawser SP et al.^[8] at Asia Pacific Region and Dissanayake et al.^[15] at Srilanka in which ESBL prevalence were 79% and 29% respectively. The our results were also not correlated with comparison studies for sensitivities of DDST and PCDDT by Priya Dutta et al.^[22] Vikas Manchanda et al.^[5] and Amita Jain et al.^[12] The antibiotic susceptibility pattern of ESBL producing Klebsiella pneumoniae in our study was slightly different from other researcher's studies in sensitive and resistant pattern of antibiotics.

SUMMARY AND CONCLUSION

The emergence and rapid spread of ESBL producing bacteria has become a worldwide problem indicating that continuous monitoring systems and effective infection control measures are absolutely required In conclusion; the present study was found 58.42% ESBL producing Klebsiella pneumoniae isolates. Most of the ESBL producing Klebsiella pneumoniae isolates were

multidrug resistant making available therapeutic choices limited. Our study also demonstrates the importance of regular review of empirical antibiotic therapy in view of the evolving resistance of ESBL producing Klebsiella pneumoniae to commonly used agents. Clinicians must depend on more laboratory guidance, while laboratories must provide resistance pattern data for optimal patient management more accurately. Additionally, improper antimicrobial use and strengthened infection control measures are required to prevent the spread and reduce the emergence of antibiotic resistance. It is essential to report ESBL production along with the routine susceptibility testing, which will help the clinician in prescribing proper antibiotics. To reduce the prevalence of antimicrobial resistant pathogens, including ESBL-producing Klebsiella pneumoniae, effective infection control measures such as hand washing and proper hospital hygiene are required. There is a need to formulate strategies to detect and prevent the emergence of ESBL producing strains for the effective treatment of infections which are caused by them. A committee must be formed at all hospitals, which should provide guidelines for the judicious use of antibiotics and should formulate policies which will help in minimizing the emergence of resistant bacteria among the patients.

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