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**Original Article**

# Study of Extended Spectrum Beta lactamase producing *Klebsiella pneumoniae* from various clinical samples at tertiary care Hospital, Jaipur

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**Abstract**
**Background:** *The present study was done to detect Extended spectrum beta lactamase producing Klebsiella pneumoniae.*
**Introduction:** *Extended spectrum of beta lactamase (ESBL) producing Klebsiella pneumoniae, frequently resistant to many of the antimicrobial agents. Antimicrobial resistance is associated with high morbidity, mortality, increased length of hospitalization and cost of health care.*
**Materials and Methods:** *A total 63 Klebsiella pneumoniae strains were isolated from various clinical specimens. Out of total 63 isolates of Klebsiella pneumoniae, total 31 ESBL Positive Klebsiella pneumoniae founded. ESBL production confirmed by double disc synergy test (DDST) and by phenotypic confirmatory disc diffusion test (PCDDT).*
**Results:** *Out of total 63 isolates of Klebsiella pneumoniae, total 31(49.20%) ESBL Positive Klebsiella pneumoniae founded. Highest prevalence of ESBL producing Klebsiella pneumoniae was observed in case of Pus 80.0%, followed by ET 58.3%, Wound Swab & Swab 50% each, Sputum 45.4 %, Blood 42.8%, and urine 37.5%. A total 31 ESBL Positive Klebsiella pneumoniae found in which 22 in Male and 09 in Female.*
**Conclusion:** *This study demonstrate that production of ESBL in Klebsiella pneumoniae is directly linked to its high antimicrobial resistance especially resistance towards aminoglycosides and cephalosporins. It is necessary to follow proper strategies to detect and prevent the emergence of resistance for appropriate and effective treatment of infections caused by Klebsiella pneumoniae.*
**Keywords:** *Klebsiella pneumoniae, ESBL, Sensitivity, Resistance.*

## Introduction

*Klebisiella pneumoniae* is an opportunistic pathogen that causes various illnesses such as urinary and respiratory tract infections and septicemia. They are Gram Negative, non motile, usually encapsulated, indole and ornithine decarboxylase negative they do not produce $H_2S$, produce lysine decarboxylase and

are generally positive in the Voges–Proskauer test. The size ranges from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length[1]. Infections due to ESBL producing *Klebsiella pneumoniae* are of concern as third generation cephalosporins are commonly used for treatment of infections due to gram negative organisms. These infections are difficult to control as they are usually associated with resistance to aminoglycosides and cephalosporins[2].

Beta lactamases are enzymes that degrade the beta-lactam ring of the beta-lactam antibiotic group such as penicillin and cephalosporins. Extended spectrum beta -lactamase (ESBL) is an acquired class A beta-lactamase that hydrolyzes and confers resistance to oxyimino second and third generation cephalosporins e.g. cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CAZ), and ceftriaxone (CTR). This is one group of beta-lactamases that is, found in certain species of Gram negative bacilli[3,4].

ESBL occur mostly among lactose fermenting members of enterobacteriaceae such as *Escherichia coli*, *Klebsiella species*, and *Enterobacter* species and rarely in non lactose fermenters like *Pseudomonas aeruginosa*. ESBLs are clinically relevant and remain an important cause of treatment failure with cephalosporins[5.]

Extended spectrum of beta lactamase(ESBL) producing *Klebsiella Pneumoniae* were first reported from Germany in 1983 and since then a steady increase in resistance against cephalosporins.

Extended spectrum of beta lactamase (ESBL) producing *Klebsiella pneumoniae*, frequently resistant to many of the antimicrobial, show significant local variations. The majority of ESBLs are derived from the widespread plasmid mediated broad-spectrum beta-lactamase TEM-1 and SHV-1, found in *Klebsiella pneumoniae* and other pathogens[6].

However, in spite of their good sensitivity to beta lactam antibiotics, there are growing concerns about increasing resistance of the organism to this same class of antibiotic because of ESBL production. In this study, we will isolate *Klebsiella pneumoniae* from different clinical samples with detection of ESBL production by different phenotypic methods and will study the antimicrobial susceptibility pattern of ESBL producing isolates.

## Materials and Methods

The prospective observational study was carried out in Department of Microbiology at National Institute of Medical Sciences & Research, Jaipur, Rajasthan. A total 63 *Klebsiella pneumoniae* strains were isolated from various clinical specimens of patients attending various outpatients and inpatients department at National Institute of Medical Sciences & Research. Samples including urine, body fluids, pus, sputum, swab etc. were processed for isolation and identification of *Klebsiella pneumoniae* within the time period of July 2018 to December 2018.

All samples were inoculated on MacConkey and Blood agar, incubated at 37°C for 24 hrs, and colonies were processed according to standard procedures. *Klebsiella pneumoniae* isolates that were obtained as a confluent growth from the clinical specimens were included in the study. The organisms were identified on the basis of colony morphology, Gram staining and biochemical reactions[7].

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by modified Kirby Bauer's disc diffusion method according to CLSI guidelines 2018. An inoculums with a turbidity equivalent to that of a 0.5 McFarland standard and Muller Hinton agar plates and commercially available antibiotics discs (Hi-Media, Mumbai) were used. Antimicrobial susceptibility testing was performed by modified Kirby Bauer's disc diffusion method according to CLSI guidelines 2018. An inoculums with a turbidity equivalent

to that of a 0.5 McFarland standard and Muller Hinton agar plates and commercially available antibiotics discs (Hi-Media, Mumbai) were used. The antimicrobial discs were used Amikacin (30µg), Ampicillin(10 µg), Cefepime (30µg), Meropenem(10µg), Ciprofloxacin (5µg), Cefotaxime(30µg), Gentamycin(10µg), Pipracillin/Tazobactam(100/10µg) , Ceftazidime(30µg), Imipenem(10µg), Ceftriaxone(30µg), Polymyxin-B (300units) and Colistin(10µg). Results were measured and recorded as compared to that of the manufacturer interpretation charts according to the Clinical and Laboratory Standard Institute (CLSI) guideline [8].

**Screening of ESBL-Producing *Klebsiella pneumoniae***

According to the CLSI guidelines, isolates showing inhibition zone of ≤ 22 mm with Ceftazidime (30µg) and ≤27 mm with Cefotaxime (30 µg) and ≤25 mm for Ceftriaxone were recorded were identified as potential ESBL producers and selected for confirmation of ESBL production using disc diffusion method.

**ESBL Confirmatory Tests**

**Double Disc Synergy Test (DDST)[9]**

The isolated colonies were inoculated in peptone water broth at 35-37°C for 2–6 hrs. The turbidity adjust to 0.5 McFarland's standard and lawn culture were made on Mueller-Hinton agar using sterile swab. Amoxicillin-clavulanic acid disc (20/10µg) placed in the centre of plate. Both side of Amoxicillin-clavulanic disc, a disc of cefotaxime (30µg) and ceftazidime (30µg), were placed at the distance of 15mm centre to centre from Amoxicillin-clavulanic acid disc. The plate were incubated at 37°C overnight. Enhancement of the zone of inhibition of third generation cephalosporin toward Amoxicillin-clavulanic acid confirmed the presence of ESBL.

**Phenotypic confirmatory disc diffusion test (PCDDT) test for ESBL detection[8]:**

A disk of Ceftazidime (30µg) alone and a disk of Ceftazidime + Clavulanic acid (30/10µg) discs were placed at least 30 mm apart, center to center. A difference of ≥5 mm between the zone diameters of Ceftazidime and its Ceftazidime/ Clavulanic acid discs is taken to be phenotypic confirmation of ESBL production.

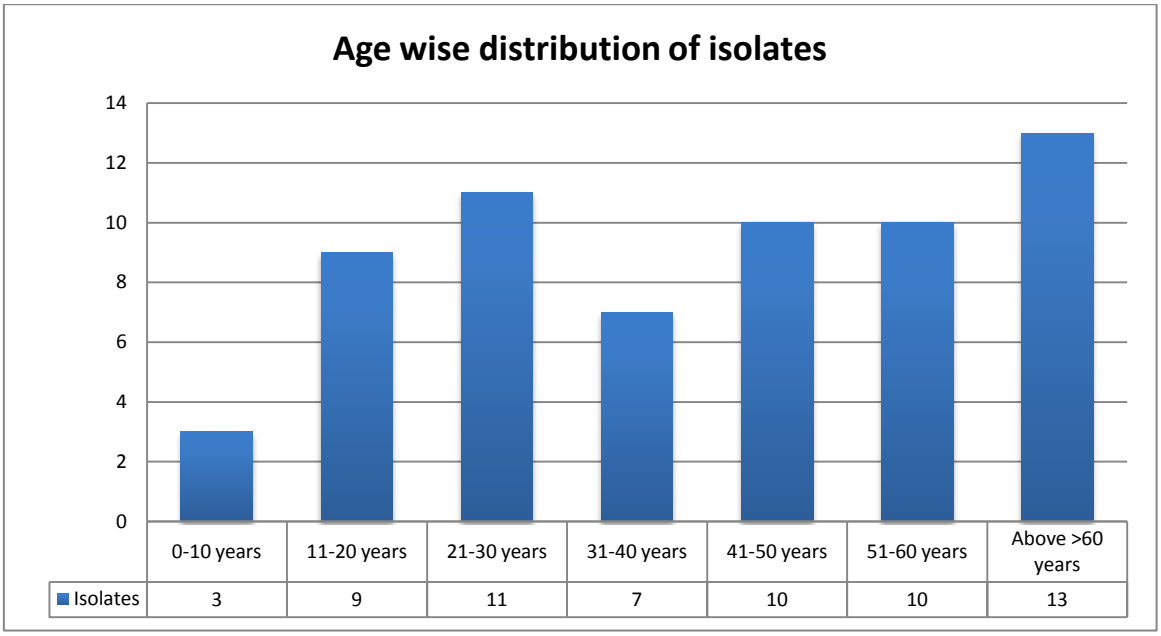
**Results**

During the study period, 63 *Klebsiella pneumoniae* were isolated from various clinical specimens of patients of all ages and both sexes at National Institute of Medical Sciences & Research. Out of total 63 isolates of *Klebsiella pneumoniae*, 42 (66.66%) isolates from male while 21 (33.33%) from female patients. Out of total 63 isolates of *Klebsiella pneumoniae*, 03(4.76%) isolated from age 0-10yrs , 11-20yrs 9(14.28%), 21-30yrs 11(17.46%), 31-40yrs 07(11.11%), 41-50 yrs 10 (15.87%), 51-60 yrs 10 (15.87%) and above 60 yrs 13 (20.63%).

Maximum sensitivity was shown with Polymyxin-B and Colistin 100% each, Meropenem 87.30%, Imipenem 66.66%, Amikacin, Gentamycin, and Pipracillin/ Tazobactam 57.14% each, Ceftazidime 47.61%, Ciprofloxacin 38.09%, Ampicillin 36.5%, Cefotaxime 31.74%. minimum sensitivity shown by Ceftriaxone 26.98%.

Out of total 63 isolates of *Klebsiella pneumoniae*, total 31(49.20%) ESBL Positive *Klebsiella pneumoniae* founded and total 32(50.79%) Non ESBL *Klebsiella pneumoniae* founded. Highest prevalence of ESBL producing *Klebsiella pneumoniae* was observed in case of Pus 80.0%, followed by ET 58.3%, Wound Swab & Swab 50% each, Sputum 45.4 %, Blood 42.8%, and urine 37.5%. A total 31 ESBL Positive *Klebsiella pneumoniae* found in which 22 in Male and 09 in Female.

**Graph 1:** Age wise distribution of *Klebsiella pneumoniae*.




**Graph 2:** Antimicrobial susceptibility pattern of *Klebsiella pneumoniae*.

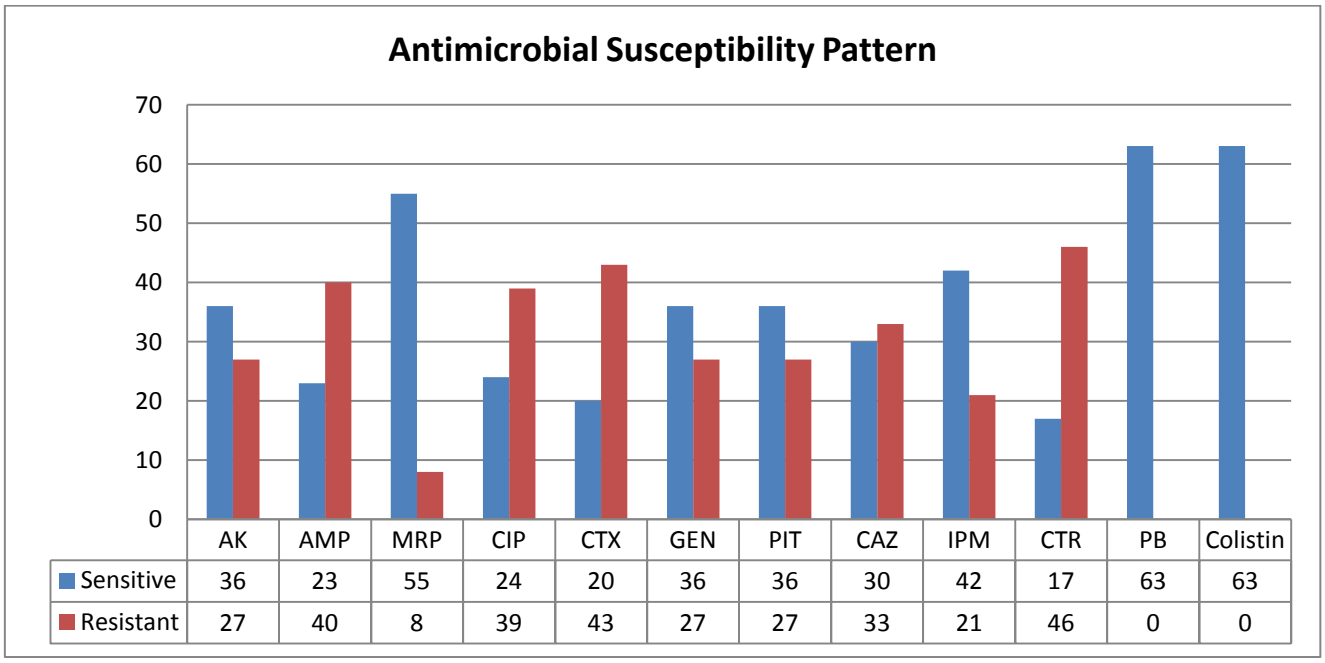



**Table: 1:** Antibiotic sensitivity pattern of *Klebsiella pneumoniae*

| S. No. | Antibiotics | Disc potency | Sensitive | Percentage % | Resistance | Percentage % |
|---|---|---|---|---|---|---|
| 1. | Amikacin | 30 µg | 36 | 57.14 % | 27 | 42.85 % |
| 2. | Ampicillin | 10 µg | 23 | 36.50 % | 40 | 63.49 % |
| 3. | Meropenem | 10 µg | 55 | 87.30 % | 08 | 12.69 % |
| 4. | Ciprofloxacin | 5 µg | 24 | 38.09 % | 39 | 61.90 % |
| 5. | Cefotaxime | 30 µg | 20 | 31.74 % | 43 | 68.25 % |
| 6. | Gentamycin | 10 µg | 36 | 57.14 % | 27 | 42.85 % |
| 7. | Pipracillin/Tazobactam | 100/10 µg | 36 | 57.14 % | 27 | 42.85 % |
| 08. | Ceftazidime | 30 µg | 30 | 47.61 % | 33 | 52.38 % |
| 09. | Imipenem | 10 µg | 42 | 66.66 % | 21 | 33.33 % |
| 10. | Ceftriaxone | 30 µg | 17 | 26.98 % | 46 | 73.01 % |
| 11. | Polymyxin-B | 300 units | 63 | 100 % | 00 | 00 % |
| 12. | Colistin | 10 µg | 63 | 100 % | 00 | 00 % |

**Graph 3 :** Total No. of ESBLs Positive *Klebsiella pneumoniae*

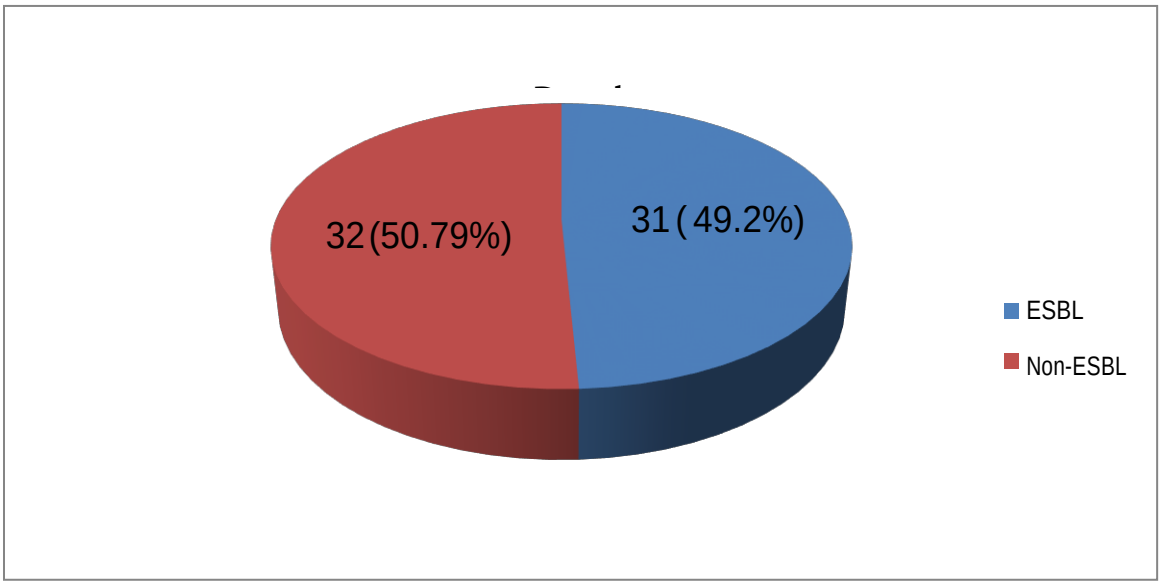
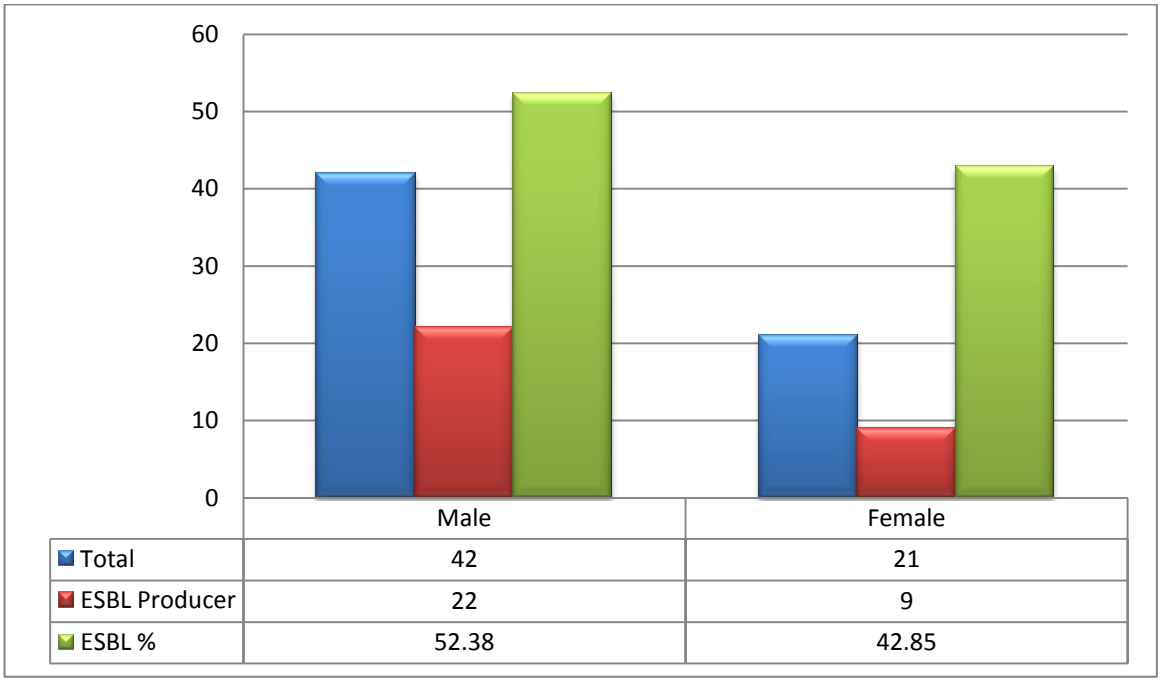



**Table 2:** Distribution of ESBLs Positive *Klebsiella pneumoniae* from various clinical sample

| S. No. | Specimen | No. of Isolates K. pneumoniae | ESBL | Percentage % | Non-ESBL | Percentage % |
|---|---|---|---|---|---|---|
| 1. | Urine | 16 | 06 | 37.5 % | 10 | 62.5 % |
| 2. | Blood | 07 | 03 | 42.85 % | 04 | 57.14 % |
| 3. | ET | 12 | 07 | 58.33 % | 05 | 41.66 % |
| 4. | Sputum | 11 | 05 | 45.45 % | 06 | 54.54 % |
| 5. | Ear Swab | 02 | 00 | 00.0 % | 02 | 100 % |
| 6. | Pus | 10 | 08 | 80.00 % | 02 | 20 % |
| 7. | Wound Swab | 02 | 01 | 50.00 % | 01 | 50.00 % |
| 8. | Swab | 02 | 01 | 50.00 % | 01 | 50.00 % |
| 9. | Semen | 01 | 00 | 00.0 % | 01 | 100 % |
| | **Total** | **63** | **31** | **49.20 %** | **32** | **50.79 %** |

**Graph 4:** Distribution of ESBL producing *Klebsiella pneumoniae* in Male and Female



**Discussion**

The present study was conducted in the Department of microbiology, National Institute of Medical Sciences & Research, NIMS University, Jaipur Rajasthan, from July 2018 to Dec 2018. During the study period, total 63 positive strains of *Klebsiella pneumoniae* were isolated from various clinical specimens at NIMS hospital. *Klebsiella pneumoniae* was common in male patients i.e. 66.66% as compare to female patients i.e. 33.33%. Similar observation of male preponderance than female was seen by Sourav Chakraborty et al 2016[10] i.e. 57% in male and 43% in female and Priyadarshini M. Deodurg, et al 2014[11] ,i.e. 56.66 % in male and 44.16 % in female. On the other hand according to Akila.K et al 2016[12], *Klebsiella pneumoniae* observed in 38.76% in male and 61.24% in female. In the present study, out of 63 *Klebsiella pneumoniae* strains isolated from different age group, the maximum no. of isolates from above 60 years old patients, i.e. 20.63% which correlate with Shristi Raut et al 2015[13] i.e. 23.5%. In the present study, out of 63 *Klebsiella pneumoniae* isolated from various clinical samples, maximum no. of isolates from urine 25.39% followed by ET 19.04%, Sputum 17.46%, Pus 15.87%, and blood 11.11%, which correlate with Akila. K et al 2016[12], i.e. Urine 52.15%, Sputum 29.67%, Pus 17.22% and Blood 0.96%. In the present study out of 63 *Klebsiella pneumoniae*, ESBL producing *Klebsiella pneumoniae* is 49.20% whereas according to Khalid Abdalla Ali Abdel Rahim et al 2014[15], No. of Klebsiella pneumoniae producing ESBL was 53.84% . In other study done by Sourav Chakraborty et al 2016[15], Klebsiella pneumoniae producing ESBL 53%. According to our study among 63 *Klebsiella pneumoniae*, ESBL producing isolates from pus were 80%, Endotracheal Tube 58.33%, Wound swab50.0%, Sputum 45.45%, Blood 42.85%, and Urine 37.5%. As compare to the study of Akila. K et al 2016[12], i.e. ESBL producing strains isolated from Urine 40.0% , Sputum 38.75%, Pus 21.25%. A study made by Faari BU et al, 2015[16], isolation rate of ESBL in *Klebsiella pneumonaie* from Swabs was 55.7% followed by Blood 17.1%, Urine 14.28%, sputum 12.85%. The prevalence of ESBLs (49.20%) in the present study was parallel from different parts of the country (14-74%). According to this study, Out of 63 isolates of *Klebsiella pneumoniae*, maximum sensitivity were against Polymyxin –B & Colistin 100% each followed by Meropenem 87.3%, Imipenem 66.66%, Amikacin, Gentamycin and Piperacillin/ tazobactam 57.14% each, Ceftazidime 47.61%, Ciprofloxacin 38.09%, Ampicillin 36.5%, Cefotaxime 31.74%, and minimum sensitivity shown by Ceftrixone 26.98%. In comparison to Khalid Abdalla Ali Abdel Rahim et al 2014[14], approximately Imipenem 84.61%, Amikacin 76.92%, Gentamycin 53.84%, Pipracillin/Tazobactam 76.92%, Ciprofloxacin 30.10%, Cefepime and Cefotaxime 23.07% each.

It has been proved that the prevalence of the ESBLs among the clinical isolates varies from country to country and institution within the same country. This might be due to judicious usage of Extended Spectrum Cephalosporins and adopting appropriate infection control measures in hospital.

**Conclusion**

This study demonstrate that production of ESBL in *Klebsiella pneumoniae* is directly linked to its high antimicrobial resistance especially resistance towards aminoglycosides and cephalosporins. This study shows that ESBL producing strains were resistant to routinely prescribed Antimicrobial agents which is an alarming sign for healthcare workers. Present study will guide the physicians to choose drug which can be used in the infection caused by the *Klebsiella pneumoniae* in our centre. Therefore it is necessary to follow proper strategies to detect and prevent the emergence of resistance for appropriate and effective treatment of infections caused by *Klebsiella pneumoniae*.

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