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# Prevalence of Extended spectrum beta- lactamase (ESBL) producing Klebsiella species and *Escherichia coli* among clinical isolates in a tertiary care hospital

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**Abstract:**

**Background**: Klebsiella species and *Escherichia coli* are members of the family Enterobacteriaceae encountered frequently as opportunistic pathogens in clinical settings. Cephalosporins are considered as first line drugs against Klebsiella species and *Escherichia coli*. Inappropriate use of β-lactams against gram negative bacteria has resulted in the widespread development of drug resistance. This study was done to know the prevalence of ESBL producing Klebsiella species and *Escherichia coli* among clinical isolates and to determine their antibiotic susceptibility patterns at Yenepoya Hospital, a tertiary care hospital.

**Materials and methods:** A total of 200 isolates were obtained from clinical samples from April to June 2016. Samples were inoculated on MacConkey's and blood agar. The organisms were identified based on standard microbiological procedures. Routine susceptibility testing was performed by Kirby Bauer's disc diffusion method, as per the recommendations of CLSI. Following screening with Cefotaxime (30 µg) disc, resistance was then confirmed for ESBL production by phenotypic confirmatory disc diffusion test using Ceftazidime (30 ug) and Ceftazidime + Clavulanic acid (30 µg + 10ug) disc.

**Results:** Out of 84 isolates of *Escherichia coli* and 116 isolates of Klebsiella species, 20(23.8%) and 26(22.4%) were ESBL producers respectively. These ESBL producers showed high degree of resistance to Ampicillin(100%) followed by Ceftazidime, Piperacillin and Cefotaxime for both Klebsiella species and *E.coli*. The most sensitive antibiotics were found to be Imipenem followed by Amikacin. Frequency distribution of ESBL producing *E.coli* and Klebsiella species was found to be highest among urine (42.5%) and sputum (25%)samples.

**Conclusion:** The presence of ESBL producers has significant implications for patient management and indicates the need for regular and routine monitoring of ESBL producing clinical isolates in laboratory practices for proper disease management.

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**Key words:** ESBL, *E.coli*, Klebsiella spp, Phenotypic onfirmatory test, Ceftazidime, Ceftazidime + Clavulanic acid.

## Introduction:

Klebsiella species *and Escherichia coli* are members of family Enterobacteriaceae, encountered frequently as opportunistic pathogens in clinical settings. The common sites for nosocomial infections caused by Klebsiella species *and Escherichia coli* include the urinary tract, lower respiratory tract, biliary tract, and surgical wound sites. People at high risk are middle-aged to older men with alcoholism, diabetes, or chronic bronchopulmonary disease. Cephalosporins are considered as first line drugs against Klebsiella species *and Escherichia coli*. Inappropriate use of β-lactams against Gram negative bacteria has resulted in the widespread development of drug resistance which is transferred among the bacteria by various genetic mechanisms.[1]

β-lactamases confer resistance to most β-lactam antibiotics. In Klebsiella spp, the β-lactamases mainly produced are AmpC and extended spectrum β-lactamases

(ESBLs). ESBLs belonging to group 2be according to Bush-
Jacoby-Medeiros classification confer resistance to penicillins, cephalosporins and monobactams but are inhibited by Clavulanic acid[2]. The present study was carried out over a period of three months from April to June 2016 in the Department of Microbiology, Yenepoya Hospital, a tertiary care hospital.
The aim of this study was to determine the antibiotic susceptibility patterns and prevalence of ESBL producing Klebsiella species and *Escherichia coli* among clinical isolates.

## Materials and Methods:

A total of 200 isolates were obtained from clinical samples from April to June 2016.

**1. Processing of Samples.**

All samples were inoculated on MacConkey's and Blood agar, incubated at 37˚C overnight, and colonies were processed. Klebsiella species *and Escherichia coli* isolates that were identified and obtained as a pure and predominant growth from the clinical specimens were only considered for the study. The organisms were identified based on standard microbiological procedures.[3]

**2. Antimicrobial Susceptibility Testing.**

Routine disc diffusion susceptibility testing was performed by Kirby Bauer's disc diffusion method. Various antimicrobial discs used included:
Cefotaxime- 30 µg; Ceftazidime- 30 µg;
Amikacin- 10 µg; Amoxycillin- 20 µg; Gentamicin- 10 µg;
Amoxyclav- 20 µg+10 µg; Imipenem- 30 µg; Ciprofloxacin- 5 µg; Piperacillin- 100µg; Piperacillin+Tazobactam- 100 µg +10 µg.
The results were interpreted as per the Clinical and Laboratory Standard Institute(CLSI) guidelines. *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) strain and *Escherichia coli* ATCC 35218 were used as control throughout the study.[4]

**3. Screening of ESBL-Producing strains for Klebsiella species and *Escherichia coli***

Clinical and Laboratory Standards Institute has developed screening tests for identifying the ESBL producers. According to CLSI guidelines, strains showing zone of inhibition of ≤ 22mm for Ceftazidime, ≤ 27mm for Cefotaxime were selected for confirmational tests of ESBL.[4]
ESBL Confirmatory Test

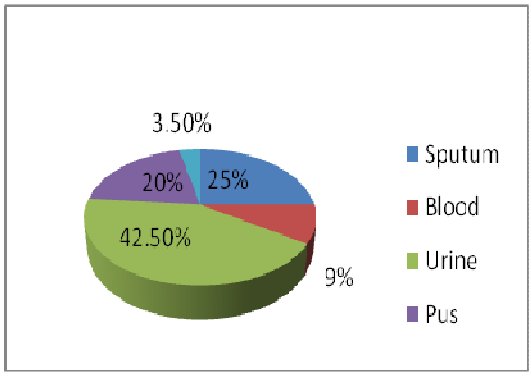
1) Phenotypic Confirmatory Disc Diffusion Test (PCDDT):

ESBL production was confirmed among potential ESBL-producing isolates by phenotypic tests. Lawn culture of the organism was made and 3rd-generation cephalosporins , Ceftazidime (30 µg) disc and Ceftazidime + Clavulanic acid (30 µg + 10µg) disc were placed 25mm apart. An increase in zone size of inhibition ≥ 5mm for Ceftazidime + Clavulanic acid compared to Ceftazidime alone confirmed ESBL production.[5]

## Results:

A total of 84 isolates of *E. coli* and 116 isolates of Klebsiella species isolated in the diagnostic Microbiology laboratory of Yenepoya Medical College, Mangalore were taken up for the study. The isolates were predominantly obtained from urine(85), sputum(50), pus (40) followed by blood(18) and others (7) (**Figure I**).

**Figure I: Distribution of organisms in clinical samples**



For the *E.coli* isolates,the most effective antibiotics were Imipenem (89.2%) followed by Gentamicin (83.3%) and Piperacillin+Tazobactum (78%). Highest resistance was seen against Ampicillin(77.3%), Piperacillin (52.3%) and Ceftazidime (48.8%) (**Table I, Figure II**).

For the Klebsiella isolates, the most effective antibiotics were Imipenem (90.5%) followed by Amikacin (78.4%) and Piperacillin + Tazobactum (77.5%).

Highest resistance was seen against Ampicillin (87.1%), Ceftazidime (54.3%) and Piperacillin (45.6%) (**Table I, Figure III**).

Among the 84 isolates of *E.coli*, 20 were identified as ESBL producers by phenotypic confirmatory disc diffusion test. Imipenem(75%), Amikacin(65%) and Amoxyclav(60%) were the most effective antibiotics against ESBL producing *E.coli*. Imipenem(82.8%), Gentamicin(76.5%) and Piperacillin + Tazobactum(71.8%) were the most effective antibiotics against non-ESBL producing *E.coli* (**Table II**).

Among the 116 isolates of Klebsiella, Imipenem (88.4%), Piperacillin + Tazobactum (73.07%) and Amikacin (57.6%) were the most effective antibiotics against ESBL producing Klebsiella. Imipenem (94.4%), Amikacin (78.8%), and Piperacillin + Tazobactum (72.2%) were the most effective antibiotics against non-ESBL producing Klebsiella (**Table III**).

In our study, the percentage of ESBL producers among *E.coli* isolates was 23.8% and Klebsiella isolates was 22.4% (**Table IV**).

The highest number of ESBL producers was found in the age group of 61-80 years: 16(34.78%) followed by 41-60years: 14(30.43%) (**Figure IV**).

## Discussion:

Klebsiella species and *Escherichia coli* remain the major ESBL producing organisms isolated worldwide which are recommended to be routinely tested and reported by the Clinical and Laboratory Standards Institute[4]. Prevalence of ESBL's varies from place to place and previous studies across India and the world over have reported ESBL production varying from 8 to 80%.

In our study, ESBL detection was carried out by the Phenotypic Confirmatory Disc Diffusion test(PCDDT) on 84 isolates of *E.coli* and 116 isolates of Klebsiella species. Imipenem was found to be the most effective (89.2%)antibiotic against *E.coli* followed by Amikacin(78.4%) and Piperacillin + Tazobactum(77.5%).

**Table I: Antibiotic sensitivity patterns of *E.coli* and Klebsiella sps among 200 isolates**

| Antibiotics | *E.coli*: 84 | | Klebsiella: 116 | |
|---|---|---|---|---|
| | Sensitive n=% | Resistant n=% | Sensitive n=% | Resistant n=% |
| Amikacin | 64= 76% | 20= 23.8% | 91= 78.4% | 25= 21.5% |
| Ampicillin | 19 = 22.6% | 65= 77.3% | 15= 12.9% | 101= 87.1% |
| Ceftazidime | 43= 51.1% | 41= 48.8% | 53= 45.6% | 63= 54.3% |
| Ceftazidime+Clavulanic acid | 55= 65.4% | 29= 34.5% | 96= 82.7% | 20= 17.2% |
| Ciprofloxacin | 52= 61.9% | 32= 38.1% | 74= 63.7% | 42= 36.2% |
| Imipenem | 75= 89.2% | 9= 10.7% | 105= 90.5% | 11= 9.4% |
| Amoxicillin+Clavulanic acid | 58= 69% | 26= 30.9% | 72= 62.1% | 44= 37.9% |
| Piperacillin | 40= 47.6% | 44= 52.3% | 63= 54.3% | 53= 45.6% |
| Piperacillin+Tazobactum | 66= 78% | 22= 22% | 90= 77.5% | 26= 22.4% |
| Gentamicin | 70= 83.3% | 14= 16.6% | 70= 60.3% | 46= 39.6% |
| Cefotaxime | 47= 55.9% | 37= 44.04% | 75= 64.6% | 41= 35.3% |

**Figure II: Antibiotic sensitivity pattern of *E.coli***

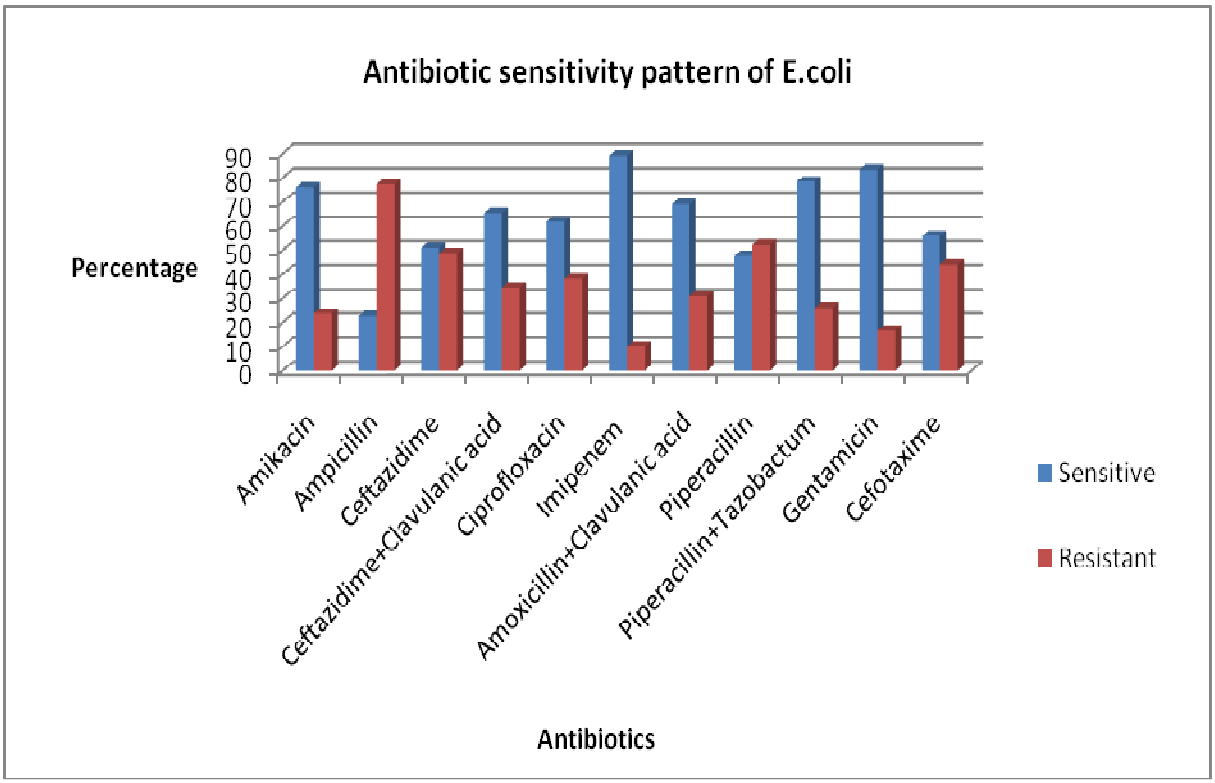



**Figure III: Antibiotic sensitivity pattern of Klebsiella sps**

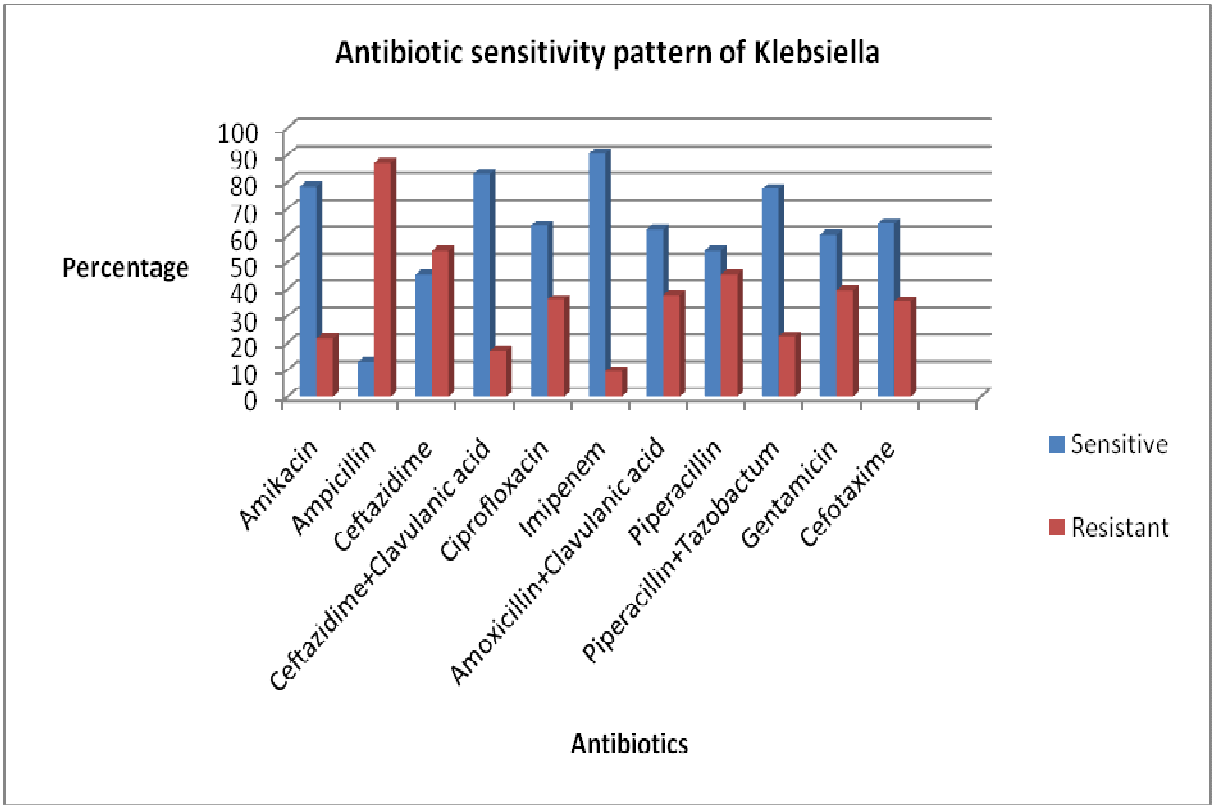


**Table II: Antibiotic sensitivity pattern of ESBL and non-ESBL producing *E.coli***

| ***E.coli* =84** | **ESBL producers= 20** | | **Non-ESBL producers= 64** | |
|---|---|---|---|---|
| **Antibiotics** | **Sensitive n=%** | **Resistant n=%** | **Sensitive n=%** | **Resistant n=%** |
| Amikacin | 13=65% | 7=35% | 40=62.5% | 24=37.5% |
| Ampicillin | 0=0% | 20=100% | 20=31.2% | 44=68.75 |
| Ceftazidime | 2=10% | 18=90% | 33=51.5% | 31=48.4% |
| Ceftazidime+Clavulanic acid | 14=70% | 6=30% | 44=68.7% | 20=31.2% |
| Ciprofloxacin | 10=50% | 10=50% | 32=50% | 32=50% |
| Imipenem | 15=75% | 5=25% | 53=82.8% | 11=17.1% |
| Amoxicillin+Clavulanic acid | 12=60% | 8=40% | 36=56.2% | 28=43.7% |
| Piperacillin | 4=20% | 16=80% | 19=29.6% | 45=70.3% |
| Piperacillin+Tazobactum | 7=35% | 13=65% | 46=71.8% | 18=28.1% |
| Gentamicin | 7=35% | 13=65% | 49=76.5% | 15=23.4% |
| Cefotaxime | 10=50% | 10=50% | 25=39.0% | 39=60.9% |

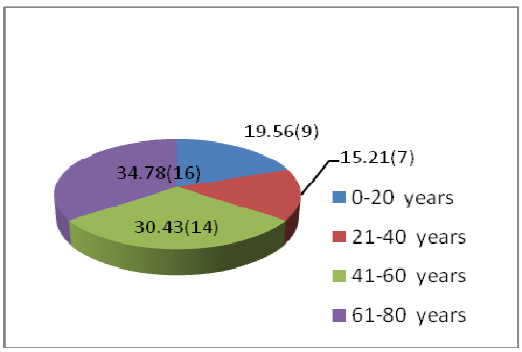
**Table III: Antibiotic sensitivity pattern of ESBL and non-ESBL producing Klebsiella sps**

| **Klebsiella= 116** | **ESBL producer=26** | | **Non-ESBL producer=90** | |
|---|---|---|---|---|
| **Antibiotics** | **Sensitive n=%** | **Resistant n=%** | **Sensitive n=%** | **Resistant n=%** |
| Amikacin | 15=57.6% | 11=42.3% | 71=78.8% | 19=21.1% |
| Ampicillin | 0=0% | 26=100% | 10=11.1% | 80=88.8% |
| Ceftazidime | 5=19.2% | 21=80.7% | 30=33.3% | 60=66.6% |
| Ceftazidime+Clavulanic acid | 22=84.6% | 4=53.8% | 76=84.4% | 14=15.5% |
| Ciprofloxacin | 12=46.1% | 14=53.8% | 49=54.4% | 41=45.5% |
| Imipenem | 23=88.4% | 3=11.5% | 85=94.4% | 5=5.5% |
| Amoxicillin+Clavulanic acid | 12=46.1% | 14=53.8% | 52=57.7% | 38=42.2% |
| Piperacillin | 12=46.1% | 14=53.8% | 43=47.7% | 47=52.2% |
| Piperacillin+Tazobactum | 19=73.07% | 7=26.9% | 65=72.2% | 25=27.7% |
| Gentamicin | 11=42.3% | 15=57.6% | 50=55.5% | 40=44.4% |
| Cefotaxime | 7=26.9% | 19=73.07% | 53=58.8% | 37=41% |

**Table IV: Percentage of ESBL and non-ESBL producing *Klebsiella* sps and *E.coli***

| **Total samples** | **200** | **ESBL producers** (46=23%) | **Non-ESBL producers** (154=77%) |
|---|---|---|---|
| Klebsiella species | 116=58% | 26=22.4% | 90=77.5% |
| *Escherichia coli* | 84=42% | 20=23.8% | 64=76.19% |

**Figure IV: Distribution of ESBL producers among age groups**




These findings correlate with a study done by Sharma et al[6] and Chika et al[7] where Imipenem was sensitive to 96.2% and 84.6% of isolates. The high sensitivity of the *E.coli* isolates in our study against Gentamycin is substantially better than that seen in studies by Khanfar et al [8]and Sarojamma et al[9]. The *E.coli* isolates in our study were most resistant to Ampicillin, Ceftazidime and Piperacillin which is similar to studies done by Sarojamma et al[9], Sharma et al[6] and Chika et al[7].

Against Klebsiella isolates, Imipenem was found to be most effective (90%)followed by Amikacin(78.4%) and Piperacillin + Tazobactum(77.5%). Studies done by Chander et al[10] showed 100% sensitivity of Klebsiella to Imipenem. However, resistance to Amikacin was only 12.5%.In our study, 20 isolates(23.8%) of *E.coli* and 26 isolates (22.4%) of Klebsiella were found to be ESBL producers.

Imipenem was the most effective antibiotic against ESBL producing *E.coli*(75%) followed by Ceftazidime + Clavulanic acid(70%) and Amikacin(65%). Against ESBL producing Klebsiella, Imipenem was most effective (88.4%) followed by Ceftazidime + Clavulanic acid(84.6%) and Piperacillin + Tazobactum(73.07%). These findings are marginally better than studies conducted by Chika et al[7] where ESBL producing Klebsiella were 26%. However, Chander et al[10] had lower ESBL rates for *E.coli*(13.5%) and Klebsiella (16.5%).

In another study done by Mumtaz S et al[11], ESBL prevalence rates were 8.94% which was much lower than other studies. The antibiotic sensitivity correlates with our study where most of the isolates are sensitive to Imipenem(95.7%) followed by Amikacin(76%).

As observed in our study, Imipenem was effective among both ESBL and non-ESBL producing *E.coli* and Klebsiella species. It should be kept in mind that Carbapenems should only be used in emergency and life threatening situations and not as a part of routine use so that the therapeutic value of these drugs is preserved and available when absolutely necessary[12].

A well designed antibiotic policy, effective implementation of the same, awareness on ESBLs in hospitals are important points to be taken into account while handling resistant organisms such as ESBL producers.

Though the prevalence rates of ESBL producers is low when compared to other studies, it is necessary to be alert to possibilities before the situation gets out of hand[13].

## Conclusion:

The presence of ESBL producers in culture isolates should be informed to the treating clinicians so that they can make an informed choice as far as appropriate antibiotics and precautions are concerned.

This will help in reducing hospital stay, save precious time and resources and also reduce the risk of spread of resistance in the hospital and community.

## References:

1.Prasanna Ramana A, Vineetha KR, Nagarathnamma T and Sathyanarayan MS. Phenotypic detection of AmpC beta-lactamase in clinical isolates of Klebsiella species by an inhibitor based method using Boronic acid.

International Journal of Current Research 2015; 7(6):17518-21.
2. Ejaz H, ul-Haq I, Mahmood S, Zafar A and Javed MM. Detection of extended-spectrum β-lactamases in *Klebsiella pneumoniae*: comparison of phenotypic characterization methods. Pak J Med Sci 2013; 29(3): 768-72; https://doi.org/10.12669/pjms.293.3576
3. Wiegand I, Geiss HK, Mack D, Stürenburg E and Seifert H. Detection of Extended-Spectrum Beta-Lactamases among *Enterobacteriaceae* by Use of Semiautomated Microbiology Systems and Manual Detection Procedures. J Clin Microbiol 2007; 45(4):1167-1174; https://doi.org/10.1128/JCM.01988-06
4. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty Second Informational Supplement. CLSI document M100- S22. Wayne, PA: CLSI; 2012.
5. Dutta H, Nath R, Saikia L. Multi-drug resistance in clinical isolates of Gram-negative bacilli in a tertiary care hospital of Assam. Indian J Med Res 2014; 139: 643-45.
6. Sharma A, Prakash MR, Veena M, Singh ER, Basavaraj KN and Vishwanath G. ESBL-A continous diagnostic challenge to clinical microbiology laboratories. IJABPT 2012; 3(2): 9-15.
7. Chika E, Moses I, Chigozie U, Peter E, Ifeanyichukwu I, Charles E. Phenotypic Detection of *Klebsiella pneumoniae* Strains - Producing Extended Spectrum β-Lactamase (ESBL) Enzymes. Sch Acad J Biosci 2013;1(1):20-23.
8. Khanfar HS, Bindayna KM, Senok AC and Botta GA. Extended spectrum beta-lactmases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: trends in the hospital and community settings. J Infect Dev Ctries 2009; 3(4): 295-99.
9. Sarojamma V and Ramakrishna V. Prevalence of ESBL producing *Klebsiella pneumoniae* isolates in tertiary care hospital. ISRN Microbiol 2011; 2011: 318348; https://doi.org/10.5402/2011/318348
10. Chander A, Shrestha CD. Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* urinary isolates in tertiary care hospital in Kathmandu, Nepal. BMC Res Notes 2013; 6: 487; https://doi.org/10.1186//1756-0500-6-487
11. Mumtaz S, Ahmed J, Ali L, Hamid H. Prevalence of extended spectrum beta lactamases(ESBL) in clinical isolates from a teaching hospital in Peshawar, Pakistan. African Journal of Microbiology Research 2011; 5(19): 2880-84; https://doi.org/10.5897/AJMR11.198
12. Manoharan A, Sugumar M, Kumar A, Jose H, Mathai D and ICMR-ESBL study group. Phenotypic and molecular characterization of AmpC β-lactamases among *Escherichia coli*, Klebsiella spp. & Enterobacter spp. from five Indian Medical Centers. Indian J Med Res 2012; 135: 359-64.
13. Arora S, Bal M. AmpC β-lactamase producing bacterial isolates from Kolkata hospital. Indian J Med Res 2005; 122: 224-33.

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