Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated at a Tertiary Care Institute in North West Region of Rajasthan, India

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**ABSTRACT**

The incidence of extended spectrum beta lactamase producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitation of therapeutic options available. Therefore we conducted this study to determine the prevalence and antibiotic susceptibility pattern of ESBL producers among *Escherichia coli* and *Klebsiella pneumoniae* isolates in the north west region of the state, as the prevalence and antimicrobial susceptibility of ESBL-producing pathogens varies from one region to another. This study was carried out in the Department of Microbiology, Sardar Patel Medical college, Bikaner from April 2009 to May 2010 on a total of 700 clinical isolates including 520 *Escherichia coli* and 180 *Klebsiella pneumoniae*. In the present study ESBL production was noticed in 65.57% isolates with maximal incidence in *Escherichia coli* (66.92%) followed by *Klebsiella pneumoniae* (61.67%) isolates. The antimicrobial resistance was significantly higher in ESBL producer than non-ESBL producer. Among ESBL producer high rate of resistance to various antibiotics was seen (cefotaxime, ceftazidime, ceftriaxone, norfloxacin, cotrimoxazole). These high rates of resistance to various antibiotics may be due to various factors like plasmid mediated transmission of ESBLs, poorly directed therapy and over the counter sales of antibiotics.

**Keywords**
ESBL, beta-lactamases, resistance, *Escherichia coli*, *Klebsiella pneumoniae*

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**Introduction**

Resistant bacteria are emerging world wide as a threat to the favourable outcome of common infections in the community and hospital settings. Beta-lactamases production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins. In the past it was believed that cephalosporins were relatively immune to attack by beta-lactamases, but latter on *Klebsiella* spp. was found to be resistant to cephalosporin. The mechanism of this resistance was production of extended spectrum beta-lactamases (ESBLs) (Chaudhary *et al.*, 2004).

Over the last 20 years, many new beta-lactam antibiotics have been developed that were
specifically designed to be resistant to the hydrolytic action of β-lactamases. However, with each new class that has been used to treat patients, new β-lactamases emerged that caused resistance to that class of the drug. Presumably, the selective pressure of the use and overuse of new antibiotics in the treatment of patients has selected for new variants of β-lactamase. One of these new classes was the oxyimino-cephalosporins, which became widely used for the treatment of serious infections due to gram-negative bacteria in the 1980s. Not surprisingly, resistance to these expanded-spectrum β-lactam antibiotics due to β-lactamases emerged quickly (Bradford, 2003).

Extended spectrum beta-lactamases (ESBLs) represent a major group of β-lactamases currently being identified worldwide in large numbers, most commonly produced by Klebsiella pneumoniae and Escherichia coli but also occur in other gram negative bacteria (Agrawal et al., 2008; Kumar et al., 2006; Rodrigues et al., 2004). ESBLs are derivative of common beta-lactamases that have undergone one or more amino acid substitution near the active site of enzyme, thereby increasing their affinity and hydrolytic activity against third generation cephalosporins and monobactams. However these plasmid mediated enzyme have no detectable activity against carbapenems (Kader et al., 2005; Shobha et al., 2009; Bishara et al., 2005; Briggs et al., 2005).

Being plasmid mediated they are easily transmitted among members of Enterobacteriaceae thus facilitating the dissemination of resistance not only to β-lactam but to other commonly used antibiotics such as quinolones and aminoglycosides.

ESBLs are specifically inhibited by β-lactamase inhibitors like clavulanic acid, and this property is commonly utilized for the detection and confirmation of ESBLs in the laboratory (Agrawal et al., 2008; Tsering et al., 2009).

As no enough study has been undertaken on prevalence and antibiotic susceptibility pattern of ESBL producing Escherichia coli and Klebsiella pneumoniae in the state of Rajasthan, with the prevalence and antimicrobial susceptibility of ESBL-producing pathogens varying from one region to another, therefore we conducted this study to determine the prevalence and antibiotic susceptibility pattern of ESBL producers among Escherichia coli and Klebsiella pneumoniae isolates in the northwest region of the state.

Materials and Methods

This study was carried out in the Department of Microbiology, Sardar Patel Medical college, Bikaner from April 2009 to May 2010 to detect Extended spectrum beta-lactamase producing strains of Escherichia coli and Klebsiella pneumoniae in a total of 700 clinical isolates. 520 Escherichia coli and 180 Klebsiella pneumoniae were isolated from various clinical specimens such as urine, blood, pus, wound, sputum and other respiratory tract specimen, body fluids, high vaginal swab, stool, semen, prostatic secretion and CSF etc. received from patients attending various outpatient departments, admitted in wards at P.B.M. hospital and associated group of hospitals.

Inclusion Criteria

All consecutive, non-duplicate isolates of Escherichia coli and Klebsiella pneumoniae were collected from various clinical specimens.
Medical and demographic data of the patients were collected using a questionnaire. Data recorded were: demographic characteristics (age, gender); underlying lung diseases, acute suppurative otitis media, diabetes mellitus, chronic renal failure, nephrotic syndrome, connective tissue disease, malignancy, immuno-compromisation, septicemia, burn, eclampsia, puerperal sepsis, pyometra, peritonitis, fractures etc. Presence of intravascular or urinary catheters; prolong hospitalization (> one week); history of intensive care unit (ICU) stay; nursing home residency; being on mechanical ventilation; prior antibiotic use; and recent surgery (within one month); poor nutritional status; haemodialysis etc. were also noted.

**Exclusion Criteria**

Isolation of three organism types with no predominating organism and repeated isolate from same patient were excluded from this study.

The samples were processed for the identification of organisms on the basis of conventional microbiological procedures and were screened for ESBLs. All isolates were cultured on Mac Conkey Agar and Blood Agar and urinary isolates on Hichrome UTI media (obtained from Hi-Media, Mumbai, India) also and incubated at 37°C for 24 hrs. They were identified to species level by their characteristic appearances on the media, Gram’s stain, Oxidase test, Motility and the pattern of the biochemical reactions. Flow chart was used for preliminary identification of organisms as shown in chart 1

Antimicrobial susceptibility of the various isolates was performed as per the Clinical and Laboratory Standards Institute (CLSI) guidelines by the Kirby Bauer disk diffusion method on Mueller Hinton agar with a 0.5 McFarland’s turbid inoculum as per CLSI recommendation (CLSI).

The zone of inhibition was measured and reported as Susceptible, Intermediate or Resistant according to standard zone size. For statistical purposes, data were categorized as susceptible and non-susceptible (including intermediate and resistant groups).

Control: *Escherichia coli* ATCC 25922 & *Staphylococcus aureus* ATCC 25923

Following antibiotic discs (obtained from Hi-Media, Mumbai, India) were used for antimicrobial sensitivity testing: Amikacin (30 µg), Amoxicillin+Clavulanic acid (20/10 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cotrimoxazole (1.25/23.75 µg), Doxycycline hydrchloride (30 µg), Gatifloxacin (5 µg), Imipenem (10 µg), Meropenem (10 µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg)

The inhibition zone diameter was measured in mm with the help of a special measuring scale and results recorded for each isolate separately as Sensitive, resistant, intermediate (S,R,I) according to the given standard zone size below for enterobacteriaceae.

Screening and confirmation of ESBL production by the phenotypic confirmatory (combination disc method) test was done as per the guidelines recommended by CLSI. Control strains, *Escherichia coli* ATCC 25922 (Beta-Lactamase negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) were used for quality control.

Initial Screening Tests (Agrawal et al., 2008; Winn et al., 2006): Inoculum was prepared by suspending few colonies of test
strain in 0.9 % sterile saline and turbidity was adjusted to 0.5 Mc Farland turbidity tube. A lawn culture was made from the inoculum using a sterile cotton swab on the surface of Mueller-Hinton agar medium and Ceftazidime (30µg) and Cefotaxime (30µg) discs were applied with all sterile precautions. The plates were incubated for 18-24 hours at 37°C.

According to the CLSI guidelines, isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg) and ≤ 27 mm with Cefotaxime (30 µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production.

Phenotypic confirmatory test with combination disc: In this test, a third-generation cephalosporin, Ceftazidime (30µg) alone and in combination with clavulanic acid (10 µg) was used. Both the discs were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured.

When there was an increase of ≥ 5 mm in inhibition zone diameter around combination disk of Ceftazidime + Clavulanic acid versus the inhibition zone diameter around Ceftazidime disc alone, was considered as confirmed ESBL producer.

Results and Discussion

Out of 700 isolates of Escherichia coli and Klebsiella pneumoniae, 459 (65.57%) were found ESBLs producers. Out of 520 Escherichia coli isolates 348 (66.92%) were found ESBLs producers and of the 180 Klebsiella pneumoniae isolates 111 (61.67%) were found ESBLs producers.

ESBL producing E. coli & K. pneumoniae strains were most frequently recovered from urine 62.36% (217/348) , 32.43% (36/111) followed by sputum & respiratory tract specimens 15.23% (53/348), 27.03% (30/111) respectively.

During past 60 years, bacteria have demonstrated a remarkable ability to resist almost every antibiotic that has been developed.(11,12,13) Extended spectrum beta-lactamases (ESBLs) represent a major group of beta-lactamases, currently being identified worldwide in large numbers, most commonly produced by Klebsiella pneumoniae and Escherichia coli.

The present study was conducted on 700 clinical isolates (520 Escherichia coli and 180 Klebsiella pneumoniae ) recovered from various clinical specimens, from all ages and both sexes attending various outpatient departments and admitted in wards at P.B.M. hospital and associated group of hospitals.

ESBL production was noticed in 65.57% isolates with maximal incidence in Escherichia coli (66.92%) followed by Klebsiella pneumoniae (61.67%). This high incidence of ESBL production agrees with Mohanty S et al., (2003) where ESBL strains were observed in 71.5% isolates (60.7% Escherichia coli and 78.7% Klebsiella pneumoniae), Rajini E et al., (2008) where ESBL production was noticed in 57% isolates (E. coli 72% followed by Klebsiella pneumoniae 38.4%), Sasirekha B et al., (2010) where 53.9% isolates were found to be ESBL producer (61.1% E.coli followed by K. pneumoniae 40.6%).

ESBL-producing E. coli strains were recovered most frequently from urine
(62.36%) followed by sputum & Respiratory tract specimens (15.23%) and pus & other wound discharges (13.79%) (Table -2). Similar observations were made by Sasirekha et al., (2010) where ESBL producing E. coli were most frequently obtained from urine (76%) followed by sputum (18.7%) and pus (5.2%), Wani K A et al., (2009) ESBL producing E. coli isolates were most commonly recovered from urine (72.9%) followed by pus 9.3% and blood 7.6% . Agarwal P et al., (2008) with ESBL-positive isolates highest among urinary isolates (70%) followed by pus (22.5%) and blood (5%).

ESBL-producing K. pneumoniae strains were recovered most frequently from urine (32.43%) followed by sputum & Respiratory tract specimens (27.03%), pus & other wound discharges (26.13%) and blood (6.31%) (table.3). Similar observations were made by Sasirekha et al., (2010) who reported maximum incidence in urine (60%) followed by sputum (28.5%) and pus (11.4%). Kusum et al., (2004) reported urine (44%) followed by sputum (42%) and blood (14%). In other studies, Waiwarawootth et al., (2006) reported higher incidence of ESBL production in sputum (44.69%), followed by urine (21.60%), pus (18.24%) and blood (10.28%) and El Astal et al., (2008) higher incidence in pus (48.4%) followed by urine (25.8%), sputum (17.2%) and blood (5.7%).

In the present study ESBL producing E. coli isolates were found to be 100% susceptible to imipenem & meropenem similar to observations made by Agarwal et al., (2008), El Astal et al., (2008), Fazlay Bazzaz et al., (2009), Wani et al., (2009) and Sasirekha et al., (2010). 60.35% ESBL producing E. coli isolates were found to be susceptible to amikacin, with variable susceptibility observed in other studies as Bishara et al., (2005) 75%, El Astal et al., (2008) 77.8% and Wani et al., (2009) 78.2%. ESBL producing E. coli isolates from urine showed 69.12% susceptibility to nitrofurantoin slightly on lower side as compared to other studies Puberza et al., (2007) 92.3% and Wani et al., (2009) 91.5% . Susceptibility to gatifloxacin was seen in 51.44 % of ESBL producing E. coli isolates while Wani et al., (2009) observed 64.1% susceptibility. 20.11% susceptibility to doxycycline hydrochloride was reported which agrees with El Astal et al., (2008) reporting 22.2% susceptibility. Cotrimoxazole susceptibility was seen in 11.21% of ESBL producing E. coli isolates with variation in other studies like Tsering DC et al., (2009) 21.52%, El Astal et al., (2008) 22.2% and Shobha et al., (2007) 42%. 32.5% ESBL producing E. coli isolates were found to be susceptible to amoxicillin+clavulanic acid similar to the studies by Bishara et al., (2005) 33%, Gupta et al., (2007) 31%, El Astal et al., (2008) 22.2%. High resistance to norfloxacin of 0.92% was seen in ESBL producing E. coli isolates from urine with other studies showing higher susceptibility. High resistance was also shown for IIIrd generation cephalosporin with only 0.86% and 0.29% ESBL producing E. coli being susceptible to ceftriaxone and cefotaxime, respectively while all strains being resistant to ceftazidime. This corresponds to study of El Astal et al., (2008) where none of the tested isolates were susceptible to cephalosporins and Wani et al., (2009) who reported 0.8%, 0.8%, 2.5% susceptibility of cefotaxime, ceftazidime and ceftriaxone respectively.

ESBL producing K. pneumoniae isolates were found to be 100% susceptible to imipenem & meropenem similar to observations made by Gupta et al., (2007), El Astal et al., (2008), Fazlay Bazzaz et al., (2009), Mehrgan et al., (2010). 55.86%
ESBL producing *K. pneumoniae* isolates were found to be susceptible to amikacin which agrees with observations made by Agarwal *et al.*, (2008) 44%, and El Astal *et al.*, (2008) 67.5%. ESBL producing *K. pneumoniae* isolates from urine showed 66.67% susceptibility to nitrofurantoin which was also observed by Mehrgan *et al.*, (2010) 60% and Puberza *et al.*, (2007) 72.2% respectively. 63.03% susceptibility to gatifloxacin was seen in ESBL producing *K. pneumoniae* isolates. 27.03% of ESBL producing *K. pneumoniae* isolates were susceptible to doxycycline hydrochloride similar to El Astal *et al.*, (2008) who reported 20% susceptibility. 5.41% susceptibility to cotrimoxazole was observed in ESBL producing *K. pneumoniae* isolates with marked variation in other studies like Brigg *et al.*, (2005) 5%, Tsering *et al.*, (2009) 21.52% and Mehrgan *et al.*, (2010) 47.1%. High resistance to amoxicillin-clavulanic acid was seen with only 2.7% of ESBL producing *K. pneumoniae* isolates being susceptible. Similar observations were made by Mehrgan *et al.*, (2010) 4.5% and Bishara *et al.*, (2005) 5% respectively. ESBL producing *K. pneumoniae* isolates were found to be 8.33% susceptible to norfloxacin similar to Shobha *et al.*, (2007) who reported 6% susceptibility. High resistance to IIIrd generation cephalosporin, with only 0.9%, 1.8% and 4.5% ESBL producing *K. pneumoniae* isolates being susceptible to cefotaxime, ceftazidime and ceftriaxone, respectively. Similar observations were made by El Astal *et al.*, (2008) where none of the tested isolates were susceptible to cephalosporins and Mehrgan *et al.*, (2010) who reported 0.6%, 1.9%, 0.6% susceptibility of cefotaxime, ceftazidime and ceftriaxone respectively while other studies showed variable higher susceptibility.

Among ESBL producer high rate of resistance to various antibiotics was seen (cefotaxime, ceftazidime, ceftriaxone, norfloxacin, cotrimoxazole). These high rates of resistance to various antibiotics may be due to various factors like plasmid mediated transmission of ESBLs, poorly directed therapy and over-the-counter sales of antibiotics.

**Association of Risk Factors with Esbl Production**

In the present study total in 459 ESBL producing isolates, 144 (31.37%) were isolated from patients with one or other type of serious illnesses followed by 112 (24.4%) in old age patients, 93 (20.26%) with prior exposure to antibiotics, 74 (16.12%) with history of recent surgery, 68 (14.81%) with invasive medical devices (urinary/arterial catheterization, central venous lines), 31 (6.75%) with poorly nourished patient (anaemia, malnutrition), 21 (4.58%) were associated with patient admitted in ICU, 09 (1.96%) with intubated and mechanically ventilated patients and 08 (1.74%) with urinary tract disease. All isolates recovered from ICU patients (21) and from intubated and mechanically ventilated (09) patients showed ESBL production.

Foley catheter, intravenous catheter, central venous catheter, intubation, surgery and mechanical ventilation were found as the risk factors for the acquisition of *E. coli* and *K. pneumoniae* with ESBLs Ozqunes *et al.*, (2006).
Chart.1 Flowchart for identification of Gram-negative lactose fermenting organisms

Growth on Mac Conkey agar (24 hours)

- Non lactose fermenters
- Lactose fermenters
- Late lactose fermenters

Motility

- Negative
- Positive

Indole test

- Positive
  - Klebsiella oxytoca
  - Klebsiella ornitholytica
- Negative
  - Klebsiella pneumoniae

Voges proskauer test

- Positive
- Negative

Lysine decarboxylase

- Positive
  - Enterobacter aerogenes
- Negative
  - Enterobacter cloacae & others
### Table 1: Distribution of ESBL Producing Escherichia coli and Klebsiella pneumoniae.

| S. No | Organism             | Total numbers | ESBL producers | Percentage (%) |
|-------|----------------------|---------------|----------------|----------------|
| 1     | *Escherichia coli*   | 520           | 348            | 66.92%         |
| 2     | *Klebsiella pneumoniae* | 180         | 111            | 61.67%         |
| **TOTAL** |                        | **700**     | **459**        | **65.57%**     |

### Table 2: Distribution of ESBL producing and non ESBL producing Escherichia coli isolates from various clinical specimens

| S.N. | Clinical sample             | Total *E.coli* isolate (%) | *E.coli* ESBL positive isolate (%) | *E.coli* ESBL negative isolate (%) |
|------|----------------------------|-----------------------------|----------------------------------|----------------------------------|
| 1.   | Urine                      | 343 (65.96%)                | 217 (62.36%)                     | 126 (73.26%)                     |
| 2.   | Sputum & Respiratory tract specimens | 59 (11.35%)                | 53 (15.23%)                      | 06 (3.49%)                      |
| 3.   | Pus & other wound discharges | 65 (12.50%)                | 48 (13.79%)                      | 17 (9.88%)                      |
| 4.   | High vaginal swab          | 21 (4.04%)                  | 11 (3.16%)                       | 10 (5.81%)                      |
| 5.   | Stool                      | 20 (3.85%)                  | 09 (2.59%)                       | 11 (6.40%)                      |
| 6.   | Body fluids                | 07 (1.35%)                  | 06 (1.72%)                       | 01 (0.58%)                      |
| 7.   | Blood                      | 02 (0.38%)                  | 01 (0.29%)                       | 01 (0.58%)                      |
| 8.   | Others                     | 03 (0.58%)                  | 03 (0.86%)                       | 00 (0.00%)                      |
| 9.   | TOTAL                      | 520                         | 348                             | 172                             |

### Table 3: Distribution of ESBL producing and non ESBL producing Klebsiella pneumoniae isolates from various clinical Specimens

| S.N. | Clinical sample             | Total *K. pneumoniae* isolate (%) | *K. pneumoniae* ESBL positive isolate (%) | *K. pneumoniae* ESBL negative isolate (%) |
|------|----------------------------|----------------------------------|-------------------------------------------|-------------------------------------------|
| 1.   | Urine                      | 75 (41.67%)                      | 36 (32.43%)                               | 39 (56.52%)                               |
| 2.   | Sputum & Respiratory tract specimens | 50 (27.78%)                      | 30 (27.03%)                               | 20 (28.99%)                               |
| 3.   | Pus & other wound discharges | 35 (19.44%)                      | 29 (26.13%)                               | 06 (8.70%)                                |
| 4.   | Blood                      | 08 (4.44%)                      | 07 (6.31%)                               | 01 (1.45%)                               |
| 5.   | Body fluids                | 05 (2.78%)                      | 04 (3.60%)                               | 01 (1.45%)                               |
| 6.   | High vaginal swab          | 04 (2.22%)                      | 03 (2.70%)                               | 01 (1.45%)                               |
| 7.   | Stool                      | 01 (0.56%)                      | 01 (0.90%)                               | 00 (0.00%)                               |
| 8.   | Others                     | 02 (1.11%)                      | 01 (0.90%)                               | 01 (1.45%)                               |
| 9.   | TOTAL                      | 180                             | 111                                         | 69                                         |
Table 4: Antimicrobial Susceptibility pattern of ESBL producing and non-ESBL producing E. coli

| S.N. | Antibiotics                      | E. coli ESBL positive isolate (%) | E. coli ESBL negative isolate (%) |
|------|----------------------------------|-----------------------------------|----------------------------------|
| 1.   | Amikacin (AK)                    | 210 (60.35%)                      | 139 (80.81%)                     |
| 2.   | Amoxicillin+Clavulanic acid (AC) | 113 (32.5%)                       | 105 (61.05%)                     |
| 3.   | Cefotaxime (CE)                  | 01 (0.29%)                        | 169 (98.26%)                     |
| 4.   | Ceftriaxone (CI)                 | 00 (0.00%)                        | 170 (98.84%)                     |
| 5.   | Ceftriaxone (CI)                 | 03 (0.86%)                        | 167 (97.09%)                     |
| 6.   | Cotrimoxazole (CO)               | 39 (11.21%)                       | 46 (26.74%)                      |
| 7.   | Doxycycline hydrochloride (DO)   | 70 (20.11%)                       | 55 (31.97%)                      |
| 8.   | Gatifloxacin (GF)                | 179 (51.44%)                      | 122 (70.93%)                     |
| 9.   | Imipenem (I)                     | 348 (100%)                        | 172 (100%)                       |
| 10.  | Meropenem (MR)                   | 348 (100%)                        | 172 (100%)                       |
| 11.  | Nitrofurantoin (NF)*             | 150 (69.12%)                      | 108 (85.71%)                     |
| 12.  | Norfloxacin (NX)*                | 02 (0.92%)                        | 32 (25.40%)                      |

*Norfloxacin and nitrofurantoin were tested against urinary isolates only.

Table 5: Antimicrobial Susceptibility pattern of ESBL producing and non-ESBL producing K. pneumonia

| S.N. | Antibiotics                      | K. pneumoniae ESBL positive isolate (%) | K. pneumoniae ESBL negative isolate (%) |
|------|----------------------------------|----------------------------------------|----------------------------------------|
| 1.   | Amikacin (AK)                    | 62 (55.86%)                            | 55 (79.71%)                            |
| 2.   | Amoxicillin+Clavulanic acid (AC) | 03 (2.70%)                             | 33 (47.83%)                            |
| 3.   | Cefotaxime (CE)                  | 01 (0.90%)                             | 68 (98.55%)                            |
| 4.   | Ceftriaxone (CI)                 | 02 (1.80%)                             | 67 (97.10%)                            |
| 5.   | Ceftriaxone (CI)                 | 05 (4.50%)                             | 65 (94.20%)                            |
| 6.   | Cotrimoxazole (CO)               | 06 (5.41%)                             | 25 (36.23%)                            |
| 7.   | Doxycycline hydrochloride (DO)   | 30 (27.03%)                            | 30 (43.47%)                            |
| 8.   | Gatifloxacin (GF)                | 70 (63.06%)                            | 57 (82.60%)                            |
| 9.   | Imipenem (I)                     | 111 (100%)                             | 69 (100%)                              |
| 10.  | Meropenem (MR)                   | 111 (100%)                             | 69 (100%)                              |
| 11.  | Nitrofurantoin (NF)*             | 24 (66.67%)                            | 34 (87.18%)                            |
| 12.  | Norfloxacin (NX)*                | 03 (8.33%)                             | 18 (46.15%)                            |

*Norfloxacin and nitrofurantoin were tested against urinary isolates only.
Table 6 Association of risk factors with ESBL production

| S.N. | Risk factors                                      | ESBL positive isolate (%) | ESBL negative isolate (%) |
|------|--------------------------------------------------|---------------------------|---------------------------|
| 1.   | Prior exposure to antibiotics                    | 93 (20.26%)               | 06 (2.49%)                |
| 2.   | ICU stay                                         | 21 (4.58%)                | 00 (0.00%)                |
| 3.   | Severe illness                                   | 144 (31.37%)              | 17 (7.05%)                |
| 4.   | Invasive medical devices (urinary/arterial catheterization, central venous lines) | 68 (14.81%) | 15 (6.22%) |
| 5.   | Intubation & Mechanical ventilation              | 09 (1.96%)                | 00 (0.00%)                |
| 6.   | Recent surgery                                   | 74 (16.12%)               | 13 (5.39%)                |
| 7.   | Old age (>60 year)                               | 112 (24.4%)               | 59 (24.48%)               |
| 8.   | Poor nutritional status                          | 31 (6.75%)                | 5 (2.07%)                 |
| 9.   | Urinary tract pathology (Polycystic kidney disease, Renal/Bladder calculi, Stricture urethra) | 08 (1.74%) | 02 (0.83%) |

Lautenbach (2001) et al., observed that the prior antibiotic use and longer duration of hospital stay were the risk factor for ESBL-producing E. coli or K. pneumoniae infection.

As ESBL-positive isolates occur in large number of patients and show false susceptibility to expanded-spectrum cephalosporins in standard disk diffusion test, therefore care should be taken by not giving cephalosporins and aztreonam, regardless of the routine susceptibility test results.

In conclusion, *Escherichia coli* (66.92%) were found to be more extended spectrum β-lactamase producer than *Klebsiella pneumoniae* (61.67%), this may be peculiar to Indian subcontinent. The antimicrobial resistance was significantly higher in ESBL producer than non-ESBL producer.Among Extended spectrum β- lactamase producing isolates imipenem and meropenem was most sensitive followed by nitrofurantoin, amikacin and gatifloxacin. Least sensitive antibiotic were cephalosporins (cefotaxime, ceftazidime, ceftriaxone). The risk factors involved in acquisition of ESBL production in the present study were:- severe illness, prior exposure to antibiotics, recent surgery, invasive medical device (urinary catheterization), poor nutritional status, ICU stay, intubation and mechanical ventilation respectively. Therefore clinician should take adequate measures in treating the patients with risk factors.

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