# Extended Spectrum B-Lactamase Mediated Resistance in Escherichia Coli in a Tertiary Care Hospital

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**Abstract:**

**Background:** ESBL occur mostly in Enterobacteriaceae e.g. Escherichia coli. ESBLs are clinically important because they destroy cephalosporin's, work horse hospital antibiotics, given as first line agents to many severely ill patients. Delayed recognition and inappropriate treatment of severe infections caused by ESBL producers with cephalosporin has been associated with increased mortality. ESBL-mediated resistance is not always obvious in vitro to all cephalosporin. Many ESBL producers are multi -resistant to non-B-Lactam antibiotics such as quinolones and amino glycosides, narrowing treatment options. Some producers achieve outbreak status spreading among patients and locals, perhaps owing to particular pathogenicity traits.

**Methods:** A total of 221 Escherichia coli isolates from different clinical specimens during the period of August 2005 to July 2007, were screened for potential ESBL activity. These strains were isolated from different clinical specimens like urine, blood, sputum, pus and other body fluids which were received in the bacteriological division of microbiology.

**Results:** Two hundred and twenty one Escherichia coli isolates were isolated from different clinical specimens like urine, blood, sputum, pus and other body fluids submitted for both in and out-patients of the hospital during the period August2005 to July 2007. All of them were subjected to screening by using ceftazidime, cefotaxime or ceftriaxone. Two hundred and eleven were positive for potential ESBL producers out of 221. 95.4%of E. coli (211/221) were screen positive which were subjected to confirmatory tests

**Conclusion:** Maximum number of ESBLs was from in-patients followed by out-patients. The out-patient presence of ESBL is of main concern as it is now come to the alert of the physician that ESBL is spreading fast in the community and responsible for community-acquired ESBLs and maximum number being from urine specimen

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## Introduction

The emergence of resistance to anti-microbial is a natural biological occurrence. The Introduction of every antimicrobial agent into clinical practice for the treatment of Infectious disease in humans and animals has been followed by the detection in the Laboratory of isolates of resistant micro-organisms i.e. micro organisms able to multiplies In the presence of drug concentrations found in hosts receiving therapeutic doses.[1]

Among antibiotics, β-lactams are the most widely used agents accounting for over 50% of all systemic antibiotics in use[2].

Mechanisms by which clinical isolate of Gram negative bacteria resist B-Lactam antibiotics are through production of Beta – Lactamase, modification of cell wall and modification of target sites with reduced affinity for Beta-Lactam antibiotics[4,5]

Among these the production of beta-lactamase appears to be of primary concern and one of the most rapidly developing and clinically significant antimicrobial resistance mechanism[3,4]

The first plasmid mediated B-lactamase in Gram negatives, TEM-1, was reported in 1965 from an Escherichia Coli isolate belonging to a patient in Athens, Greece, named Temnoniera (hence the designation TEM.). Another common plasmid mediated β -lactamase found in Klebsiella pneumoniae and Escherichia coli is SHV-1 (named after the sulfhydryl "variable"active site)[7]. Newer beta-lactam antibiotics (extended spectrum β-lactam) that would not be susceptible to these enzymes become widely used in the 1980s[4]. The first report of plasmid-encoded B-lactamase capable of hydrolysing the extended spectrum cephalosporins was published in 1983.[7] which was first isolated in Germany, ESBLs spread rapidly to Europe, US and Asia and are now found all over the world. Being plasmid mediated, they are easily transmitted among members of enterobacteriaceae thus facilitating the dissemination of resistance not only to Beta-lactams but to other commonly used antibiotics such as quinolones and aminoglycosides.[4]

E. coli is one of the most common isolate in our hospital settings and as B-lactam antibiotics are mainstay of treatment, the increasing number of E. coli isolates exhibit ESBLs; and as such B-lactam group of antibodies will be almost ineffective in few years to come.. E. coli being commonest organism after Klebsiella to exhibit ESBLs, complicates the problem, unless a definitive policy of detecting such ESBL producing isolates and their in time reporting to clinicians is communicated, so that appropriate treatment is instituted and the resulting morbidity and mortality are substantially reduced.

Objective: To study the prevalence of ESBL producing E. coli isolated from different clinical specimen and the drug resistance pattern of ESBL producing E. coli.

## Methods

### *Clinical isolate*

A total of 221 Escherichia coli isolates from different clinical specimens during the period of August 2005 to July 2007, were screened for potential ESBL activity. These strains were isolated from different clinical specimens like urine, blood, sputum, pus and other body fluids which were received in the bacteriological division of microbiology. All the samples were processed and identified as per the standard bacteriological division of microbiological [9].

E .coli ATCC 25922 was used as a negative ESBL control. Based on routine antibiotic disk sensitivity tests, isolates that exhibited resistance to any one of the third generation cephalosporin, ceftazidime /cefotaxime were shortlisted to detect and confirm ESBL producers.

### *Antibiotics*

The following antibiotic sensitivity disks were used for primary screening ceftazidime30µg, cefotaxime30µg and ceftriaxone 30µg. In addition, Augmentin disk containing 20µg of amoxicillin plus 10µgof clavulanic acid and ceftazidime + cavulanic acid were used for confirmatory tests. E-test strips of ceftazidime and ceftazidime + clavulanic acid was used for selected ESBL isolates.

### *Screening for ESBLs by double disk synergy test*

Escherichia coli that exhibited resistance to third generation cephalosporin's were screened to detect ESBL producers. Cefotaxime 30µg was placed at a distance of 15mm edge to edge from a centrally placed augmentin disk containing 20µg of amoxicillin+10µg of clavulanic acid.Plates were incubated at 35$^{o}$c for 18-20 hours and the pattern of zones of inhibition was noted. Isolates that exhibited a distinct shape/size with potentiating towards amoxicillin+clavulanic disk were considered potential ESBLproducers [14, 15, 18,19,20,21].

***Phenotypic confirmation disk diffusion test***

ESBL production was confirmed among potential ESBL producing isolates by phenotypic tests. Third generation cephalosporins with and without clavulanic acid were used as follows – ceftazidime 30µg (ca) and ceftazidime (30µg) + clavulanic acid (10µg). *Disk diffusion assay was carried out as per guidelines of CCLS and difference in zone* diameters between disk with and without clavulanic acid were recorded[14, 15, 18, 19].

***E-test of selected ESBL isolates***

E-test of select ESBL isolates was carried out. Ratio of ceftazidime MIC and ceftazidime + clavulanic acid MIC equal to or greater than 8 indicated the presence of ESBL[14, 16].

## Results

Two hundred and twenty one Escherichia coli isolates were isolated from different clinical specimens like urine, blood, sputum, pus and other body fluids submitted for both in and out-patients of the hospital during the period August2005 to July 2007. All of them were subjected to screening by using ceftazidime, cefotaxime or ceftriaxone

Two hundred and eleven were positive for potential ESBL producers out of 221.

95.4%of E. coli (211/221) were screen positive which were subjected to confirmatory tests (Fig. 1).

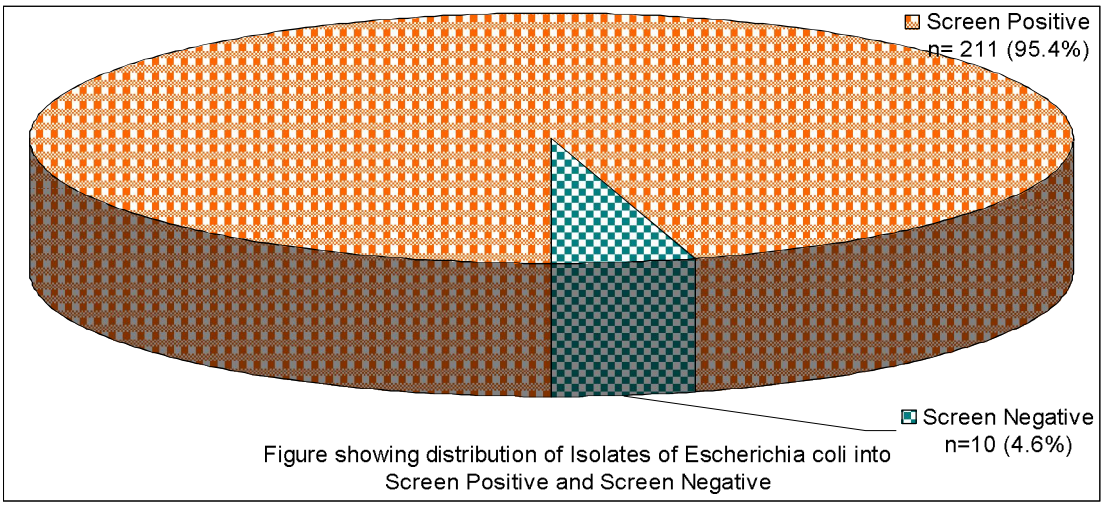


**Fig. (1). Out of 221 *Escherichia coli* isolates, 211 (95.4%) were detected as Screen positive whereas 10 (4.6%) did not show any ESBL production.**

**Table (1). Age and Gender wise distribution of ESBL positive *Escherichia coli* isolates.**

| Age & Gender | | Positive | | Negative | | Result |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Age | 0 to 9 | 12 | 10.2 | 7 | 7.5 | 0.577(NS) |
| | 10 to 19 | 1 | .8 | 3 | 3.2 | |
| | 20 to 29 | 14 | 11.9 | 14 | 15.1 | |
| | 30 to 39 | 38 | 32.2 | 20 | 21.5 | |
| | 40 to 49 | 18 | 15.3 | 16 | 17.2 | |
| | 50 to 59 | 17 | 14.4 | 19 | 20.4 | |
| | > or = 60 | 18 | 15.3 | 14 | 15.1 | |
| Gender | Male | 45 | 38.1 | 34 | 36.6 | 0.814(NS) |
| | Female | 73 | 61.9 | 59 | 63.4 | |

As shown in Table (1), maximum number of ESBL producers belonged to age group 30-39 yrs.i.e. 38 (32.2%).

Overall male female ratio of 1:16 was observed which was not statistically significant when compared to ESBL negative isolates of Escherichia -Coli

Fig. (2) PCDDT detected 99.2 %( 117/118) E. coli positive for ESBL production. 10 isolates among 117 potential ESBL producers were also confirmed by DDST.

Table (2) shows that distribution of ESBL positive isolates was highest among wards, 11.9% from nephrology, 8.5% from gastroenterology and general medicine each while least number were isolated from hematology 2%, oncology 2.5% and neurology 3 %.

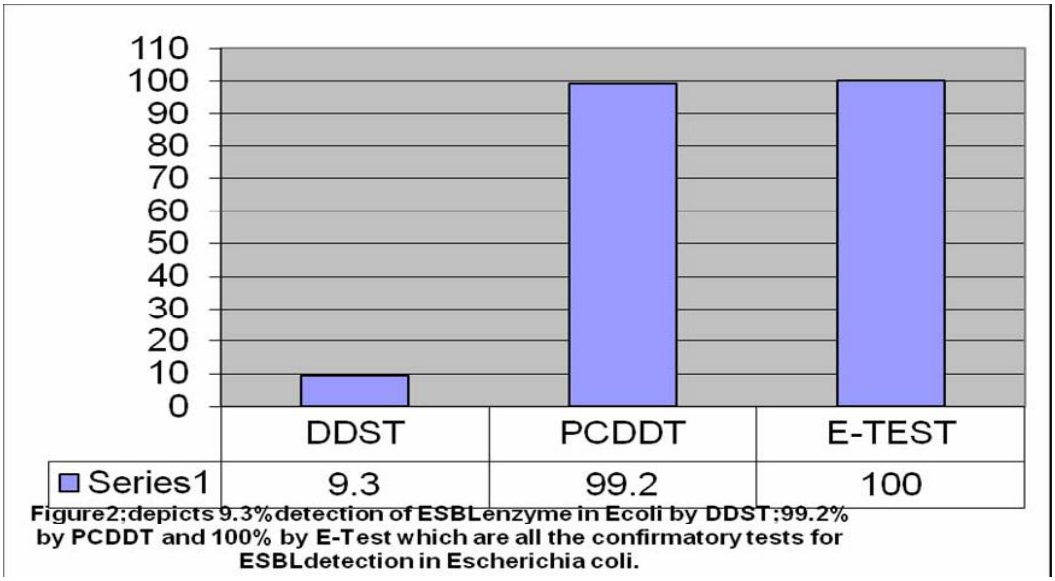


**Figure2;depicts 9.3%detection of ESBLenzyme in Ecoli by DDST;99.2% by PCDDT and 100% by E-Test which are all the confirmatory tests for ESBLdetection in Escherichia coli.**

*Twenty selected isolates were confirmed by E-test method. All of them tested positive for ESBL production with MIC's ranging from 42.66µg/ml to320µg/ml (fig4.pic4). MIC's of 256µg/ml,128µg/ml and 64µg/ml was obtained for each four selected ESBL isolates respectively while MIC's of 84.21µg/ml and 42.66µg/ml was obtained for each two selected ESBL isolates. For two isolates MIC's was 168µg/ml while one isolate had MIC' of 320µg/ml*

**Table (2). Ward wise distribution of ESBL positive *Escherichia coli* isolate.**

| Age & Gender | | Positive | | Negative | | Result |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| Ward | Cardiology | 5 | 4.2 | 2 | 2.2 | 0.864(NS) |
| | CVTS | 4 | 3.4 | 1 | 1.1 | |
| | Neurosurgery | | | 1 | 1.1 | |
| | Accident & Emergency | 4 | 3.4 | 5 | 5.4 | |
| | General Surgery | 6 | 5.1 | 3 | 3.2 | |
| | Gastroenterology | 10 | 8.5 | 6 | 6.5 | |
| | Urology | 5 | 4.2 | 7 | 7.5 | |
| | Nephrology | 14 | 11.9 | 9 | 9.7 | |
| | General Medicine | 10 | 8.5 | 9 | 9.7 | |
| | Endocrinology | 8 | 6.8 | 4 | 4.3 | |
| | Plastic Surgery | 2 | 1.7 | 9 | 9.7 | |
| | Neurology | 3 | 2.5 | 3 | 3.2 | |
| | Neonatology | 5 | 4.2 | 5 | 5.4 | |
| | Hematology | 2 | 1.7 | 2 | 2.2 | |
| | Oncology | 3 | 2.5 | 1 | 1.1 | |
| | OPD | 34 | 28.8 | 25 | 26.9 | |
| | SICU | 3 | 2.5 | 1 | 1.1 | |

Table (3) shows that ESBL positive strains were isolated from almost 84% patients with a stay more than one week while only 16.7 were isolated from patients with a stay less than one week. This is statistically insignificant when compared with ESBL negative strains 87.3% positive ESBL patients had a history of prior use of third generation cephalosporin.

**Table (3). Hospital Stay in patients with ESBL positive isolates of *Escherichia coli*.**

| Hospital Stay in patients with ESBL positive isolates of *Escherichia coli* | | | | | |
|---|---|---|---|---|---|
| **Hospital stay** | **Positive** | | **Negative** | | **Result** |
| | **n** | **%** | **n** | **%** | |
| < or = one Week | 14 | 16.7 | 8 | 11.8 | 0.394(NS) |
| > one Week | 70 | 83.3 | 60 | 88.2 | |

**Table (4). Antibiotic susceptibility of ESBL Producers and Non-producers of *Escherichia coli*.**

| Antibiotic susceptibility of ESBL Producers and Non-producersof *Escherichia coli* | | | | | |
|---|---|---|---|---|---|
| **Antibiogram** | | **ESBL** | | | |
| | | **Positive** | | **Negative** | |
| | | **n** | **%** | **n** | **%** |
| Ce_Cephalosporins | Sensitive | 1 | 0.8 | | |
| | Resistant | 117 | 99.2 | 93 | 100.0 |
| Ca_Cephalosporins | Sensitive | 1 | 0.8 | | |
| | Resistant | 117 | 99.2 | 93 | 100.0 |
| Ci_Cephalosporins | Sensitive | 3 | 2.5 | | |
| | Resistant | 115 | 97.5 | 93 | 100.0 |
| C+S_Cephalosporins | Sensitive | 76 | 80.0 | 38 | 55.1 |
| | Resistant | 19 | 20.0 | 31 | 44.9 |
| Cefpime | Resistant | 14 | 100.0 | 15 | 100.0 |
| Amikacin | Sensitive | 43 | 78.2 | 32 | 59.3 |
| | Resistant | 12 | 21.8 | 22 | 40.7 |
| Gentamicin | Sensitive | 31 | 34.8 | 14 | 21.5 |
| | Resistant | 58 | 65.2 | 51 | 78.5 |
| Ciprofloxacin | Sensitive | 8 | 6.9 | 9 | 10.7 |
| | Resistant | 108 | 93.1 | 75 | 89.3 |
| Ofloxacin | Sensitive | 4 | 3.8 | 5 | 6.2 |
| | Resistant | 102 | 96.2 | 76 | 93.8 |
| Gatifloxacin | Sensitive | 41 | 64.1 | 41 | 68.3 |
| | Resistant | 23 | 35.9 | 19 | 31.7 |
| Levofloxacin | Sensitive | | | 2 | 66.7 |
| | Resistant | 2 | 100.0 | 1 | 33.3 |
| Moxifloxacin | Sensitive | | | 2 | 50.0 |
| | Resistant | 10 | 100.0 | 2 | 50.0 |
| Nitrofurantoin | Sensitive | 54 | 91.5 | 15 | 53.6 |
| | Resistant | 5 | 8.5 | 13 | 46.4 |
| Cotrimaxozole | Sensitive | 29 | 30.9 | 16 | 23.9 |
| | Resistant | 65 | 69.1 | 51 | 76.1 |
| Imipenem | Sensitive | 58 | 98.3 | 3 | 15.0 |
| | Resistant | 1 | 1.7 | 17 | 85.0 |
| Meropenem | Sensitive | 8 | 88.9 | 7 | 100.0 |
| | Resistant | 1 | 11.1 | | |

*Ci= ceftriaxone, Ce=cefotaxime, Ca=Ceftazidime, C+S=Cephalosporin+Salbactum*

Third generation cephalosporin's showed 97.5% to 99.2% resistance while cephalosporin + clavulanic acid combination reported only 19% resistance in ESBL producer. In quinolones; levofloxacin and moxifloxacin reported 100% resistance followed by ofloxacin and ciprofloxacin 96.2% and 93.1% respectively.

In aminoglycosides, gentamicin showed 65.3% resistance.Cotrimaxozole resistance was seen in 69.1% isolates.Imipenem, nitrofurantoin, meropenam, gatifloxacin and amikacin showed 98.3%, 88.9%, and 64.1% and 78.2% sensitivity respectively.

In Non-ESBL producer's 100% resistance seen among third and fourth generation cephalosporin. In quinolones, ciprofloxacin had 89%, ofloxacin 93% resistance.

In amino glycosides, gentamicin had 78.8% resistance. Cotrimaxozole had 68% resistance.

Table (5) shows that Urine (72.9%) was the main source of ESBL's production in all the specimen followed by pus 9.3% and blood 7.6%

**Table (5). Isolation of ESBL positive *Escherichia coli* from various clinical Specimens.**

| **Isolation of ESBL positive *Escherichia coli* from various clinical Specimens** | | | | | |
|---|---|---|---|---|---|
| | **Positive** | | **Negative** | | |
| Specimen | n | % | n | % | Result |
| Urine | 86 | 72.9 | 59 | 63.4 | |
| pus | 11 | 9.3 | 16 | 17.2 | |
| Pleural fluid | 2 | 1.7 | | | |
| Blood | 9 | 7.6 | 8 | 8.6 | |
| Bile | 6 | 5.1 | 9 | 9.7 | |
| Asitic fluid | 1 | .8 | 1 | 1.1 | |
| Endotracheal tip | 1 | .8 | | | |
| Sputum | 1 | .8 | | | |
| CSF | 1 | .8 | | | 0.207(NS) |
| | | | | | |

## Discussion

The present study was conducted with above perspective in view to identify the potential ESBL producers which were subjected to confirmatory tests by Double Disk Synergy test (DDST), phenotypic Confirmatory Disk Diffusion test (PCDDT) and *E-test* to know the prevalence of ESBL positive strains. Percentage detection by confirmatory tests was obtained and resistance pattern to third generation cephalosporin's and other antibiotics was observed.

In the present study, total number of Escherichia coli isolates subjected to screening was 221. Among them 211 was screen positive for potential ESBL production that was further subjected to confirmatory test.

Criterion for selection of ESBL producing strains was done by CLSI recommendations for screening tests initially followed by confirmatory tests [5, 12,13]. Cefpodoxime and Ceftazidime have been proposed by NCCLS (now CLSI) as indicator of ESBL production as compared to Cefotaxime and Ceftriaxone. Cefpodoxime and Ceftazidime show the highest sensitivity for ESBL screening. However the use of more than one of the five antimicrobial drugs suggested for screening will improve the sensitivity of detection.[7,13] 95.4% of the Escherichia coli isolates (211/221), (Fig 1) were screen positive using above mentioned three disks. These screen positive isolates (i.e. 211) were further subjected to confirmatory tests.

In the present study, higher percentage of resistance to Ceftazidime (99.2%), Cefotaxime (99.2%) and Ceftriaxone (99.5%) was seen and use of more than two screening agents for screening increased the incidence of potential ESBL producers in screening test. Amit Jain et al [10] reported resistance to Cefotaxime of more than 80.9% and upto 59.5% to Ceftazidime.– Akbar M. Rafay et al [9] observed 100% resistance to Cephalosporins – Ceftazidime, cefotaxime, Ceftriaxone. –Bithika Duttaroy and Suchi Mehta et al (3) showed resistance of 75% to Cefotaxime, 85% to Ceftazidime and 60% to Ceftriaxone .

The present study showed 11 Escherichia Coli isolates out of 128 potential ESBL producers to be confirmed ESBL positive strains by Double Disk Synergy test which is 9.3% (11/118) (Fig2) and is very much less when compared to other studies. The reasons for discordance are various factors like precise placement of discs, correct storage of the clavulanic disks and performance of appropriate control tests are critical to the sensitivity of DDST. Double disk test can lack sensitivity because of the problems of optimal discs spacing and the inability of the test to detect ESBLs in strains producing chromosomal cephalosporinases.[3,16,20]

Emergy *et al* [21] from Virgina made observations while conducting study of detection and clinical significance of extended-spectrum B-lactamases in a tertiary care medical centre. By the DDST, ESBL production was detected in only 1.5% of isolates of the family enterobacteriaceae and in only 1.2% of patients tested. A similar low prevalence of 1.5% was reported by Sirot et al in a survey of French hospital. The reasons for such low number of DDS in our study and above mentioned study are the choice of drugs tested and the distance between disks which varied from study to study. Most important, DDST does not detect all ESBL-producing isolates.[21] While double disk potentiating test was a simple and convenient method to detect ESBLS, a phenotypic confirmatory test is recommended by NCCLs (now CLSI) is mandated to confirm the presence of ESBL. [4]

PCDDT (Phenotypic confirmatory disk diffusion test) detected 117 (99.3%), (Fig 2) potential ESBL producers to be ESBL positive by this confirmatory test. Ten among 117 potential ESBL producers were also confirmed by DDST.

Linscott et al from louisiana and Detriot Michigen evaluated four commercially available extended spectrum βeta-lactamases phenotypic confirmatory tests. [13] The test sensitivity and specificity of confirmatory test (PCDDT) were 96% and 100% respectively in concordance with our current study of 99.3% (22I) .In the current study, E-test confirmation test method detected ESBL production in twenty selected ESBL confirmed by other confirmatory tests. Their ESBL status and MIC was determined. MIC's of ≥8 fold was considered positive for ESBL positive isolates. [6]

All the twenty selected ESBL isolates were tested positive by E-test strips with different MIC's ranging from 42.66 µg/ml to 320 µg/ml. MIC's of 128 µg/ml, 256 µg/ml and 64 µg/ml was obtained for each four selected ESBL isolates respectively while MIC's of 84.21 µg/ml and 42.66 µg/ml was obtained for each two selected ESBL isolate. For two isolates MIC's was 168 µg/ml while one isolate had MIC's of 320 µg/ml. So, 100% results were obtained by this method in our current study, which is comparable to various studies. The reported sensitivity of this method as phenotypic confirmatory test for ESBLs is 87%to 100% and specificity is 95%to 100% which depends upon the ratio of MIC's of the cephalosporin verses cephalosporin/clavulanate combination used (the manufacturer currently recommends a ≥8 fold reduction in cephalosporin MIC's in the presence of clavulanate) [7]

In the current study it was observed that maximum no. of ESBL positive isolates were from Inpatients 71.1% (84/118) followed by 28.8% (34/118) from outpatients which matched with a study reporting ESBL producing bacteria 87% from inpatients and 12.7% from outpatients by Abdulrahman A Kader et al [17]

The distribution of ESBL positive isolates was highest among wards i.e ,11.8% from Nephrology, 8.4% from Gastroenterology and 8.4% from General Medicine while least number were isolated from Hematology 2%, Oncology 5% and Neurology 3%.Urine (72.8%)was the main source of ESBL's production in all the specimen followed by pus 9.3% and blood 7.6% which was almost similar to study by Bithikia *et al* [3] in which ESBL strains isolated from all the wards of the hospital were mainly recovered from urine (49.05%)followed from pus (30.18%). Much higher (58%) prevalence of ESBL producers in urinary isolates of gram negative bacilli was observed in India by Mathur et al [11] Most of the patients with ESBL positive strains had diagnosis of urinary tract infection (27.9%) followed by sepsis 17.7% and other medical diagnosis.

Akbar M. Rafay *et al* in Oman hospital reported majority of ESBLs from medical wards (29.6%) followed by patients who attended out patients (24.3%) clinics at Sultan Qaboos University Hospital [12] Urine (70.4%) was the main source of ESBLs from all patients. This high percentage of ESBLs from outpatient clinics and accident and Emergency should alert the physician.

Rodriquez-Bano *et al* in a recent nationwide study of ESBL producing organisms in Spain reported 93% of ESBL-producing strains were isolated from inpatients while 51% of ESBL producing E. coli (ESBLEC) strains from outpatients [12]

The prevalence of ESBL positive Escherichia coli among potential ESBL was 55.9% (118/211) producers confirmed by DDST, PCDDT and E test .The above percentage of ESBL positive isolates is in

concordance with various studies and varies among different geographical areas, countries and institutions.

Amit Jain *et al* [10] detected marked geographic variation. Incidence in India was 47.5% and other countries 36% to 55%. A study from Northern India [10] in 2000 by Amit Jain reported an incidence of 58.06% for ESBL producing Escherichia coli which is almost equal to above current study conducted. S B Padmini et al [11] detected majority of ESBL producers among patients admitted in medical ICU and surgical ward and production was found to be 41% in Escherichia coli .Ali AM et al [22] detected the frequency of Escherichia coli ESBL positive strains as 48% which was being the most frequent ESBL producers .A study by Bithika Duttaroy et al [3] found that 46.5% of Escherichia coli to be ESBL positive by DDST and MIC reduction test. When compared with the current study of 55.9%, which is higher percentage, is due to use of three confirmatory tests for ESBL detection, which increased the prevalence percentage.

## Conclusion

It is concluded from the above observation of the current study that maximum number of ESBLs was from in-patients followed by out-patients. The out-patient presence of ESBL is of main concern as it is now come to the alert of the physician that ESBL is spreading fast in the community and responsible for community-acquired ESBLs and maximum number being from urine specimen

ESBL occur mostly in Enterobacteriaceae e.g. Escherichia coli. ESBLs are clinically important because they destroy cephalosporin's, work horse hospital antibiotics, given as first line agents to many severely ill patients. Delayed recognition and inappropriate treatment of severe infections caused by ESBL producers with cephalosporin has been associated with increased mortality. ESBL-mediated resistance is not always obvious in vitro to all cephalosporin. Many ESBL producers are multi -resistant to non-B-Lactam antibiotics such as quinolones and amino glycosides, narrowing treatment options. Some producers achieve outbreak status spreading among patients and locals, perhaps owing to particular pathogenicity traits.

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