

*Full Length Research Paper*

# Extended spectrum - $\beta$ -lactamase mediated resistance in *Escherichia coli* in a tertiary care hospital in Kashmir, India

Kaiser Ahmed<sup>1</sup>, Manzoor A. Thokar<sup>2\*</sup>, Abubaker S. Toboli<sup>3</sup>, Bashir A. Fomda<sup>2</sup>, Gulnaz Bashir<sup>2</sup> and Peer Maroof<sup>2</sup>

<sup>1</sup>Government Medical College, Srinagar Kashmir India.

<sup>2</sup>Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Kashmir, India.

<sup>3</sup>Department of Microbiology and Parasitology, Faculty of Medicine, Garunis University, Benghazi, Libya.

Accepted 11 November, 2010

To study the prevalence of extended spectrum  $\beta$ -lactamase (ESBL) producing *Escherichia coli* isolated from different clinical specimens received in the Department of Microbiology, Sher-i-Kashmir institute of Medical sciences (SKIMS) Kashmir, India and to observe the drug resistance pattern of these ESBL producing *E. coli*. Various isolates of *E. coli* were obtained from patients admitted or attending Out Patient Department (OPD) over a period of 2 years from 1st August, 2005 to 31st July, 2007. In this study, 221 *E. coli* were subjected to screening by using cefotaxime, ceftazidime and ceftriaxone 30  $\mu$ g discs. Among them, 211 were screen positive for potential ESBL productions which were further subjected to confirmatory tests by phenotypic methods: Double Disc Synergy Test (DDST), Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and E-test. 55.9% (118/211) of *E. coli* isolates were positive for ESBL production from different clinical specimens, maximum number being from urine (72.9%). The maximum number of ESBL producing isolates were from inpatients (71.2%) followed by outpatient (28.8%). Resistance pattern of ESBL positive isolates showed resistance to 3rd and 4th generation cephalosporins (97.5 to 99.2%), quinolones (93.1 to 100%) and aminoglycosides (65.2%) in that order respectively. They showed high degree of sensitivity to imipenam (98.3%), nitrofurantoin (91.5%), gatifloxacin (64.1%) and amikacin (78.2%). Higher prevalence of ESBL production in *E. coli* of almost 55.9% should alert the physicians. Presence of ESBL producing isolates in outpatients is of main concern as it shows fast spreading ESBL problem in our community. Multidrug resistance pattern of ESBL isolates was studied in detail.

**Key words:** *Escherichia coli*, double disc synergy test, phenotypic confirmatory disc diffusion test, extended spectrum  $\beta$ -lactamase.

## INTRODUCTION

Resistant bacteria are a fast spreading global threat both in community and hospital settings (Chaudhary and Aggarwal, 2004). Among antibiotics,  $\beta$ -lactams are the most widely used agents accounting for over 50% of all systemic antibiotics currently in use (Kumar et al., 2006). Mechanisms by which Gram negative bacteria produce resistance to  $\beta$ -lactam antibiotics are  $\beta$ -lactamase

enzyme induced, modification of cell wall and modification of target sites with reduced affinity for  $\beta$ -Lactam antibiotics (Duttaroy and Mehta, 2005; Ghatole et al., 2004). Among these the production of  $\beta$ -lactamase appears to be of primary concern and one of the most rapidly developing and clinically significant antibiotic resistance mechanism (Duttaroy and Mehta, 2005; Ghatole et al., 2004). First plasmid mediated  $\beta$ -lactamase in Gram negatives was reported in 1965 from an *Escherichia coli* isolate belonging to a patient in Athens, Greece, named Temnoniera (hence designated TEM). Another common plasmid mediated  $\beta$ -lactamase found in *Klebsiella pneumoniae* and *E. coli* is SHV-1 (named after

\*Corresponding author. E-mail: manzoorthakur@rediffmail.com, manzoorthakur@gmail.com. Tel: 2187654535.

the sulfhydryl "variable" active site) (Al-Jasser, 2006).

There was a major effort in the past two to three decades to design new  $\beta$ -lactam antibiotics that would not be hydrolysed by these inactivating enzymes and third generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime were used successfully in such situations then.

Concept that these newer  $\beta$ -lactam antibiotics (also known as extended spectrum  $\beta$  lactam group) would not be susceptible to these enzymes in 1980's (Duttaroy and Mehta, 2005) remained short lived. The first report of plasmid-encoded  $\beta$ -lactamase capable of hydrolysing the extended spectrum cephalosporins was published in 1983 (Al-Jasser, 2006) which was first reported from Germany. ESBL producing microbes have spread rapidly since then in Europe, USA and Asia and are now prevalent all over the world (Kumar et al., 2006).

Being plasmid mediated, they are easily transmitted and disseminate resistance not only to  $\beta$ -lactams but also to other commonly used antibiotics such as quinolones and aminoglycosides (Kumar et al., 2006).

*E. coli* is one of the most common isolates in our hospital settings from different specimens and  $\beta$ -lactam antibiotics remaining the mainstay of treatment, huge number of *E. coli* isolates exhibiting ESBL production are rendering these  $\beta$ -lactam group of antibiotics defunct and ineffective in such situations.

*E. coli* is the commonest organism after *Klebsiella* to exhibit ESBL production and as no study has been done on *E. coli* before at SKIMS, so current study was undertaken to study the prevalence and resistance patterns of clinical isolates of ESBL producing *E. coli* by employing Double Disk Synergy Tests (DDST), Phenotypic Confirmatory Disk Diffusion Tests (PCDDT) and E-test.

## METHODS

### Clinical isolates

A total of 221 *E. coli* isolates from different clinical specimens from August, 2005 to July, 2007, were screened for potential ESBL production. Strains were isolated from different clinical specimens such as urine, blood, sputum, pus and other body fluids which were received in the bacteriology division of the department. All samples were processed and identified as per the standard bacteriological procedures (Collee et al., 1999). *E. coli* ATCC 25922 were used as a negative ESBL control.

### Criterion for selection of ESBL producing strains

Routine disc diffusion susceptibility testing of these isolates was performed by modified Kirby Bauer method following the Clinical Laboratory Standard Institute (CLSI) criteria with one of the third generation cephalosporins. Isolates found to be resistant or with decreased susceptibility to any of the third generation cephalosporins such as ceftazidime, cefotaxime or ceftriaxone were shortlisted for ESBL testing (Duttaroy and Mehta, 2005; Paterson and Bonomo, 2005). Ceftazidime 30  $\mu$ g, cefotaxime 30  $\mu$ g and ceftriaxone 30  $\mu$ g discs were used for screening purpose. In addition, augmentin disk containing 20  $\mu$ g of amoxicillin plus 10  $\mu$ g



Figure 1. Double disc synergy test.

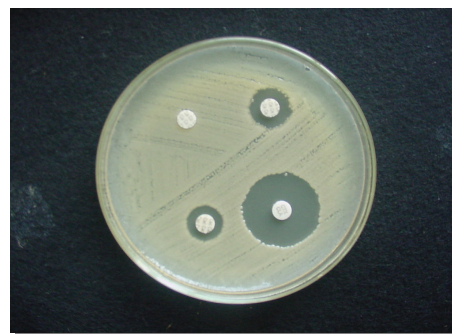


Figure 2. Phenotypic confirmation disk diffusion test.

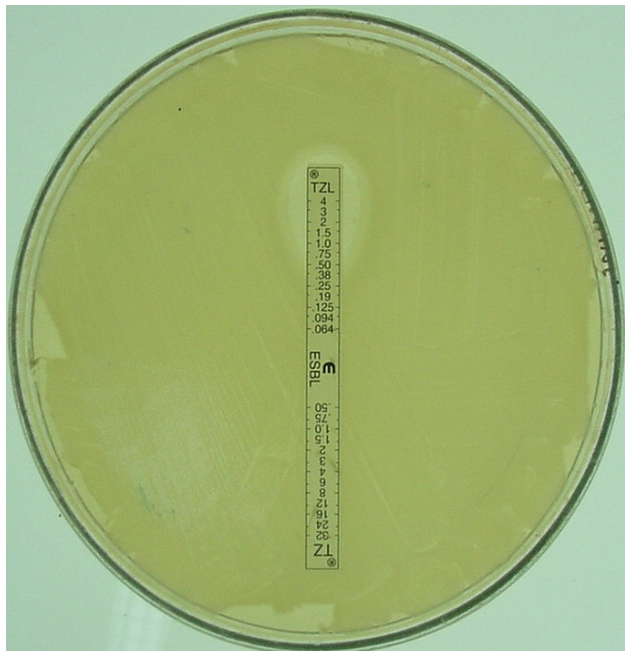
of clavulanic acid and ceftazidime + clavulanic acid were used for confirmatory tests. E-test strips having the concentration gradient of ceftazidime and ceftazidime + clavulanic acid on either sides were used to confirm the ESBL isolates.

### Screening for ESBLs by double disc synergy test

*E. coli* that exhibited resistance to third generation cephalosporins were screened to detect ESBL production by DDST. Cefotaxime 30  $\mu$ g was placed at a distance of 15 mm edge to edge from a centrally placed augmentin disk containing 20  $\mu$ g of amoxicillin + 10  $\mu$ g of clavulanic acid. Plates were incubated at 35°C for 18-20 h and the pattern of zone inhibition was noted. Isolates that exhibited a distinct shape/size with potentiation towards amoxicillin + clavulanic disc were considered potential ESBL producers (D'Azevedo et al., 2004; Emery and Weymouth, 1997) Figure 1.

### Phenotypic confirmation disk diffusion test [D'Azevedo et al., 2004; Jenny, ???; Luzzano et al., 2006; Andrea and William, 2005]

ESBL production was confirmed by phenotypic tests. Third generation cephalosporins with and without clavulanic acid such as ceftazidime (30  $\mu$ g) and ceftazidime (30  $\mu$ g) + clavulanic acid (10  $\mu$ g) were used in the study Figure 2. Disk diffusion assay was carried out as per CLSI guidelines (Paterson and Bonomo, 2005) and difference in zone diameters between disk with and without



**Figure 3.** E-test.

clavulanic acid were recorded (D'Azevedo et al., 2004; Jenny, ???; Luzzano et al., 2006; Andrea et al., 2005).

#### **E-test of selected ESBL isolates [D'Azevedo et al., 2004; Villanueva et al., 2003]**

E-test of only some selected ESBL isolates was carried out and ratio of ceftazidime Minimum Inhibitory Concentration (MIC) and ceftazidime+clavulanic acid MIC equal to or greater than 8 indicated a confirmed ESBL isolate. Only few isolates could be subjected to E test because of shortage of E test testing material (D'Azevedo et al., 2004; Villanueva et al., 2003) (Figure 3).

## **RESULTS**

Two hundred and twenty one *E. coli* isolates were isolated from different clinical specimens such as urine, blood, sputum, pus and other body fluids from both inpatients and out-patients from August, 2005 to July, 2007. All of the isolates were subjected to screening by using ceftazidime, cefotaxime or ceftriaxone 30 µg discs. Two hundred and eleven isolates were screen for positive potential ESBL producers out of 221 tested isolates (95.4%) which were then subjected to confirmatory tests (Figure 4).

One hundred and eighteen (118) isolates of *E. coli* tested positive for ESBL production by three confirmatory tests such as DDST, PCDDT and E-Test. Double disk synergy test showed only eleven *E. coli* isolates positive for ESBL production( 9.3%)-(Figure 5 and Figure 1).

PCDDT confirmed 99.2% (117/118) *E. coli* isolates positive for ESBL production (Figure 2 and Figure 5). Ten isolates among 117 potential ESBL producers were also

confirmed by DDST. Twenty selected isolates were confirmed by E-test method. All of them tested positive for ESBL production with MIC's ranging from 42.66 to 320 µg/ml (Figure 5 and Figure 3).

The maximum no of 71.1% ESBL positive isolates were from inpatients (71.1%) followed by 28.8% from outpatient (Figure 6 and Table 1). Majority of inpatients were young adults belonging to active reproductive age group and majority of ESBL producing *E. coli* were from urine, pus and blood in that order respectively.

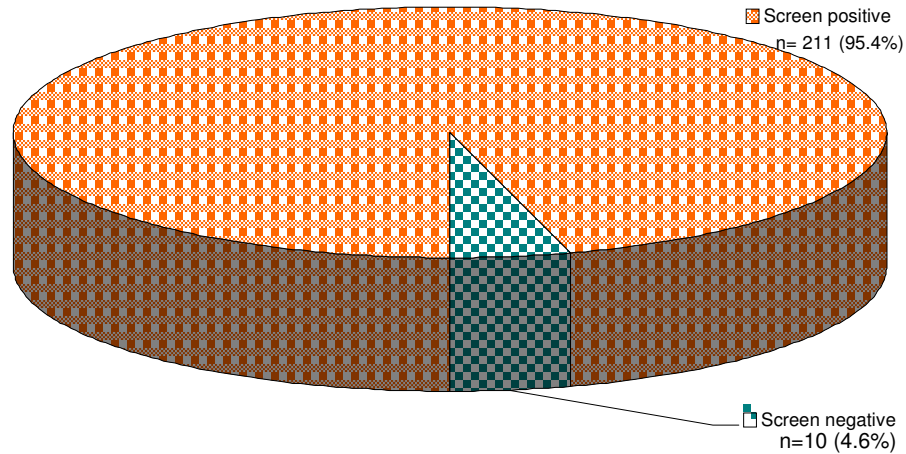
Distribution of ESBL positive isolates was highest among inpatients such as 11.8% (nephrology), 8.4% (gastroenterology and general medicine), 2% (haematology), 5% (oncology) and 3% (neurology) (Table 1). Urinary isolates were maximum with 72.9% age positivity followed by pus (9.3%) and blood (7.6%) (Table 2). Percentage positivity of ESBL producing *E. coli* among potential screen positive ESBL producers was 55.9% (118/211) which was also confirmed by PCDDT and E-test. Third generation cephalosporins showed 97.5 to 99.2% resistance while cephalosporin + clavulanic acid combination depicted only 20% resistance in ESBL producers (Table 3 and Figure 7). Fourth generation cephalosporine cefepime showed 100% resistance. Levofloxacin and moxifloxacin reported 100% resistance followed by ofloxacin and ciprofloxacin 96.2 and 93.1% respectively. Gentamicin showed 65.3% resistance whereas cotrimoxazole resistance was seen in 69.1% isolates. Imipenem, nitrofurantoin, meropenam, gatifloxacin and amikacin depicted 98.3, 91.5, 88.9, 64.1 and 78.2% sensitivity respectively (Table 3 and Figure 8). In non ESBL producers 100% resistance was seen for third and fourth generation cephalosporins (Table 3).

## **DISCUSSION**

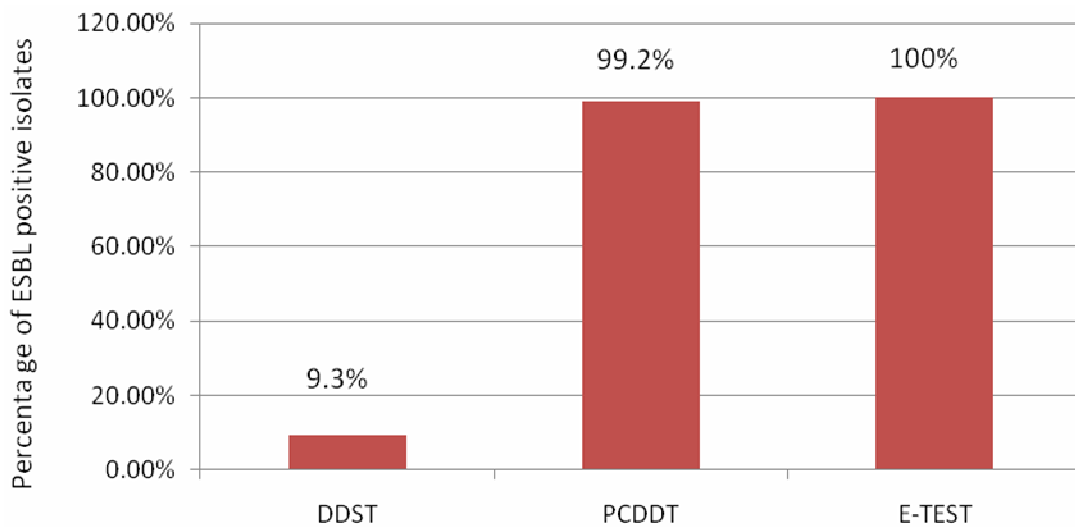
ESBL producing *E. coli* are clinically important because they hydrolyse 3rd generation of cephalosporins, which are given as first line treatment agents to severely ill patients. Delayed laboratory diagnosis and inappropriate treatment results in increased morbidity and mortality. ESBL-mediated resistance is not always obvious *in vitro* conditions to these cephalosporins. Many ESBL producing microbes are multi drug resistant to non-β-lactam antibiotics also such as quinolones and aminoglycosides thus narrowing treatment options.

Some ESBL producing Gram negative bacteria produce outbreaks as they spread fast among patients and in the community (Ali et al., 2004). Present study was conducted to identify potential *E. coli* ESBL producers which were subsequently subjected to confirmatory tests by DDST, PCDDT and E-test to know the prevalence as well as resistance pattern of these ESBL positive *E. coli* strains.

Selection of ESBL producing strains was done by CLSI recommendations such as initially for screening tests



**Figure 4.** Out of 221 *E. coli* isolates, 211 (95.4%) were detected as screen positive whereas 10 (4.6%) did not show any ESBL production.



**Figure 5.** Depicts 9.3% of ESBL producing *E. coli* by DDST; 99.2% by PCDDT and 100% by E-Test which are all the confirmatory tests for ESBL detection in *E. coli*.



**Figure 6.** Positive DDST and PCDDT simultaneously on the same culture plate showing ESBL production in *E. coli*.

followed by confirmatory tests (Al-Jasser, 2006; Yunsong et al., 2002; Ahmed and Salam, 2002). In this study, 95.4% of the *E. coli* isolates were screen positive using cefotaxime, ceftazidime and ceftriaxone 30 µg each discs as initial screening agent which were further subjected to confirmatory tests. The 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 chance of diagnosing ESBL producers in screening as well as confirmatory tests and this is in agreement with various studies conducted in India and abroad (Duttaroy and Mehta, 2005; Rafay et al., 2007; Jain et al., 2003).

ESBL positive strains confirmed by DDST 9.3% is very much less when compared to other studies. DDST can

**Table 1.** Ward wise distribution of ESBL positive *E. coli* isolates.

Ward	Positive		Negative	
	n	%	n	%
Cardiology	5	4.2	2	2.2
CVTS	4	3.4	1	1.1
Neurosurgery	0	0	1	1.1
Accident and 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
Haematology	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 2.** Showing isolation of ESBL positive *E. coli* from various clinical specimens.

Specimen	Positive		Negative	
	N	%	n	%
Urine	86	72.9	59	63.4
Pus	11	9.3	16	17.2
Pleural fluid	2	1.7	0	0
Blood	9	7.6	8	8.6
Bile	6	5.1	9	9.7
Asitic fluid	1	0.8	1	1.1
Endotracheal tip	1	0.8	0	0
Sputum	1	0.8	0	0
CSF	1	0.8	0	0

also lack sensitivity because of the problems of optimal discs spacing (Duttaroy and Mehta, 2005; Villanueva et al., 2003; Vercauteren et al., 1997) and our result was comparable with another study conducted at a tertiary care centre in India (Emery and Weymouth, 1997). PCDDT result is also in agreement with lot more other studies in India and abroad (Luzzano et al., 2006; M'Zali et al., 2000). E-test confirmation test detected ESBL production in twenty selected ESBL positive isolates (100%) which were positive by other screening and confirmatory tests as well (Rodrigues et al., 2004; Tenover et al., 1999).

Maximum number of ESBL *E. coli* positive isolates were from inpatients followed by outpatients which is in accordance with lot other studies and depicting ESBL *E. coli* producers in 87% of cases in inpatients and 12.7%

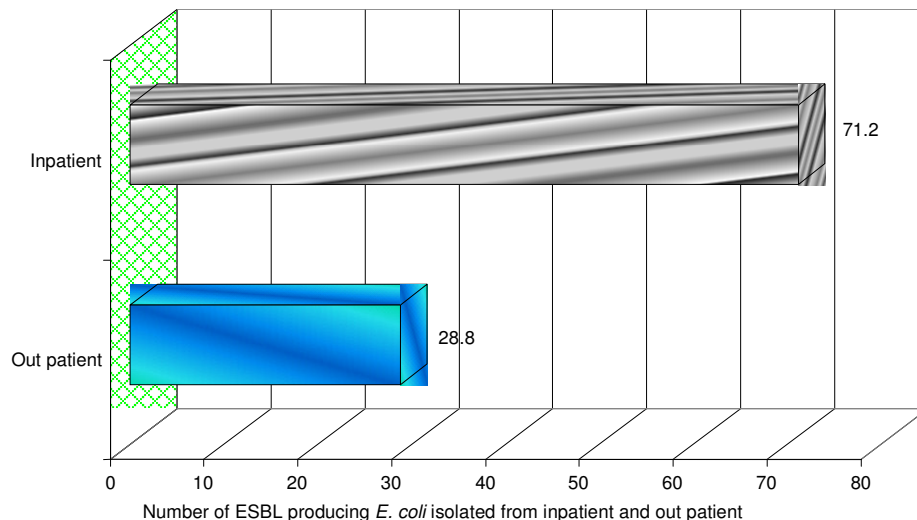
from outpatients (Kader and Angamuthu, 2005). This high percentage of ESBLs from inpatients and outpatients should alert the physicians. Urine was the main source of ESBL producing *E. coli* in the specimens followed by pus and blood which was almost similar to studies by Duttaroy and Mehta (2005) and Akbar M. Yunsong et al., 2002. Much higher (58%) percentage positivity of ESBL producers in urinary isolates was observed in India by Padmini and Appalaraju, 2004.

Presence of ESBL producing *E. coli* in the community is of main concern as ESBLs are spreading fast in the community all over the globe (Chaudhary and Aggarwal, 2004; Luzzano et al., 2006; Andrea et al., 2005). The percentage positivity of ESBL positive *E. coli* of 55.9% (118/211) was confirmed by DDST, PCDDT and E-test. The above prevalence of ESBL positive isolates is in

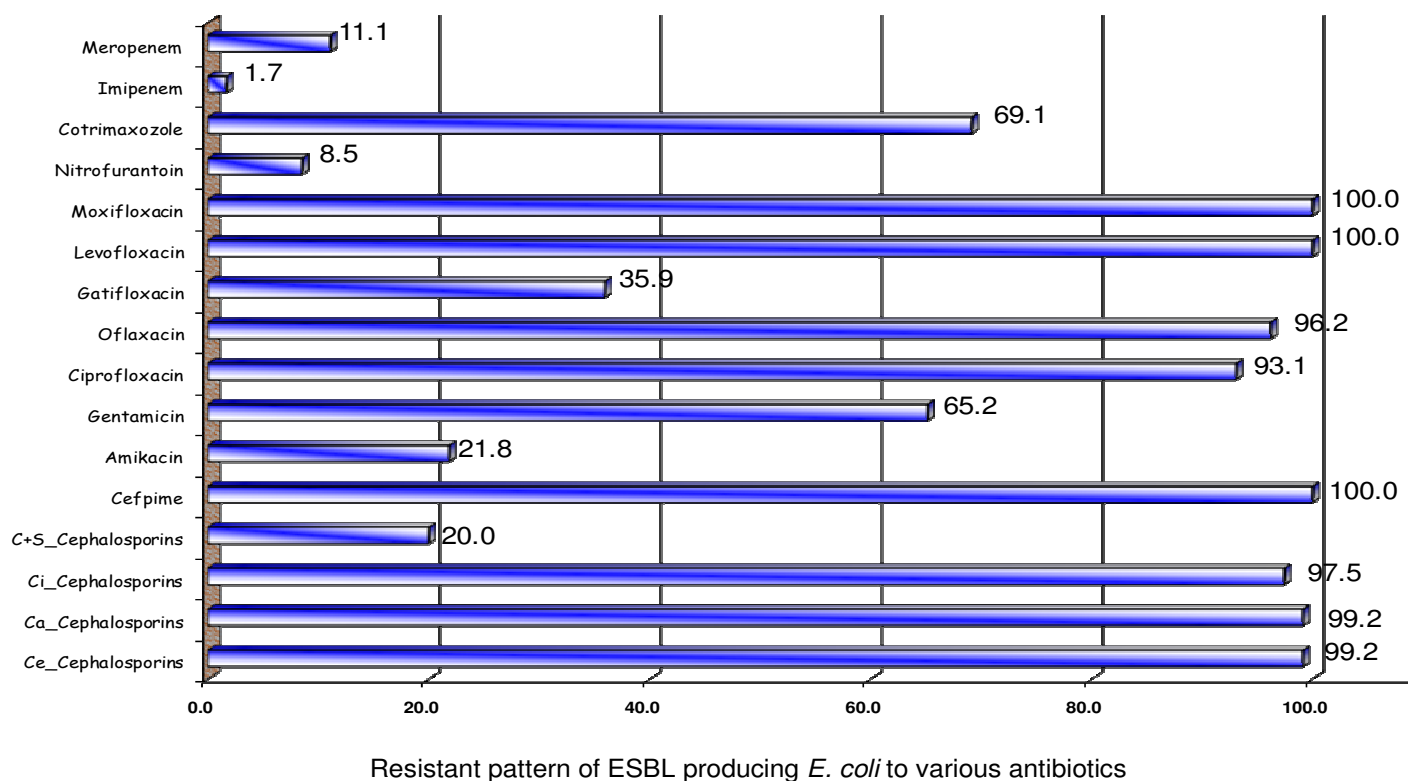
**Table 3.** Antibiotic susceptibility of ESBL Producers and non-producers of *E.coli*.

Antibiogram	ESBL				
	Positive	Negative			
		n	%	n	%
*Ce_Cephalosporins	Sensitive	1	0.8	0	0
	Resistant	117	99.2	93	100
**Ca_Cephalosporins	Sensitive	1	0.8	0	0
	Resistant	117	99.2	93	100
***Ci_Cephalosporins	Sensitive	3	2.5	0	0
	Resistant	115	97.5	93	100
#C+S_Cephalosporins	Sensitive	76	80.0	38	55.1
	Resistant	19	20.0	31	44.9
Cefpime	Resistant	14	100	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	0	0	2	66.7
	Resistant	2	100	1	33.3
Moxifloxacin	Sensitive	0	0	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
Cotrimoxazole	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
	Resistant	1	11.1	0	0

\*Cefotaxime, \*\*ceftazidime, \*\*\*ceftriaxone, #cefotaxime plus sulbactam.



**Figure 7.** Depicts distribution pattern of ESBL positive *E. coli* isolated from outpatients that is 34 (28.8%) and inpatients 84 (71.2%).



**Figure 8.** Showing antibiogram of ESBL producing *E. coli*.

concordance with various studies and sometimes they vary from institutions, countries and different geographical areas (Duttaroy and Mehta, 2005; Jain et al., 2003; Padmini and Appalaraju, 2004; Ali et al., 2004).

Amit-Jain et al. (2003) have reported marked geographic variation as incidence in India was seen in

47.5% isolates and other countries depicted percentage positivity of 36 to 55%. A study from Northern India (Amit-Jain et al., 2003) in 2000 by Amit Jain reported an incidence of 58.06% for ESBL producing *E. coli* which is in agreement to our current study. The frequency of ESBL-producing organisms differs significantly as per

geographic location. Although, frequency of ESBL producing *E. coli* in Europe, North Latin America and Western Pacific is reported between 1-8%, its prevalence in the Asia Pacific region and South Africa is reported at more than 20% (Jabeen et al., 2005). The lower prevalence of ESBL in western countries compared to others can be explained by vigorous implementation of infection control measures, restricted and judicious use of oxyimino-cephalosporins, induction of timely need based ESBL detection methods recommended by CLSI, importance of hand hygiene reinforcement, isolation precautions of patients colonized or infected with ESBL producers, continuous medical education programs and research studies which all contribute to minimize the spread of ESBL production.

Antibiogram of both  $\beta$ -lactam and non- $\beta$ -lactam group of antibiotics showed multidrug resistance pattern (Table 3 and Figure 8). Tankhiwala et al. (2004) reported 82% resistance to cotrimoxazole in her study from India. Nitrofurantoin constitutes a reasonable option for treatment (62.5%) sensitivity and Amikacin can be a drug of choice as 85.7% isolates were sensitive to this drug. Both these drugs showed similar results in our study as well.

Cephalosporin plus sulbactam showed 20% resistance which was higher than reported in another study (Yunsong et al., 2002) and a study by Faith D. reported multidrug resistance pattern similar to our study but reported 100% sensitivity to imipenem and meropenam. In contrast our study showed 11.1% resistance for meropenam (Yunsong et al., 2002; Rafay et al., 2007; Kader and Angamuthu, 2005; Padmini and Appalaraju, 2004; Tankhiwala et al., 2004).

ESBL producing organisms hydrolyse  $\beta$ -lactam antibiotics, so antibiotic choice for infections with such organism is vastly reduced. Further, the plasmids bearing the genes encoding ESBLs frequently carry genes encoding resistance to aminoglycosides and trimethoprim/sulfamethoxazole (Paterson and Bonomo, 2005). In our study multidrug resistance is observed for 3rd and 4th generation cephalosporins, quinolones and aminoglycosides. Once an ESBL producing strain is detected, the laboratory should report it as "resistant" to all penicillins, cephalosporins and aztreonam, even if they are sensitive *in vitro* conditions. Other anti-microbial agents should be reported as per their antibiogram (Samaha-Kfoury and Araj, 2003). ESBL producing bacteria are a global threat and as we have presently increase in resistance to carbapenems also, this should be taken as a great concern they were considered as last resort treatment in patients admitted in intensive care units and high risk wards (Gupta et al., 2006).

## REFERENCES

- Ahmed I, Salam A (2002). Extended spectrum beta-lactamases and bacterial resistance. Pak. J. Med. Sci., 18(2): 151-155.
- Ali AM, Rafi S, Qureshi AH (2004). Frequency of extended spectrum  $\beta$ -Lactamase producing gram negative bacilli among clinical isolates at clinical laboratories of Army Medical College, Rawalpindi. J. Ayub Med. Coll Abbottabad., 16(1): 135-137.
- Al-Jasser AM (2006). Extended-spectrum  $\beta$ -lactamases (ESBL's): Global problem. Kuwait Med. J., 38(3): 171-185.
- Andrea JL, William JB (2005). Evaluation of Four commercially available extended-spectrum  $\beta$ -Lactamase phenotypic confirmatory tests. J. Clin. Microbiol., 43(3): 1081-1085.
- Chaudhary U, Aggarwal R (2004). Extended spectrum  $\beta$ -lactamases (ESBL)-an emerging threat to clinical therapeutics. Ind. J. Med. Microbiol., 22(2): 75-80.
- Collee JG, Miles RS, Walt B (1999). Tests for the identification of bacteria in 'Mackie and McCartney as editors. Practical Medical Microbiology, Ed. fourteenth, Elsevier. Pub Churchill Hill Livingstone., pp. 131-150.
- D'Azevedo PA, Goncalves ALS, Musskopf MI, Ramos CG, Dias CAG (2004). Laboratory tests in the detection of extended spectrum  $\beta$ -Lactamase production: National Committee for Clinical Laboratory Standards (NCCLS) screening test, the E-test, the double disk confirmatory list, and cefoxitin susceptibility testing. Braz. J. Infect. Dis., 8(5): 372-377.
- Duttaroy B, Mehta S (2005). Extended spectrum  $\beta$ -lactamases (ESBL) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. Indian J. Pathol. Microbiol., 48(1): 45-48.
- Emery CL, Weymouth LA (1997). Detection and clinical significance of extended spectrum  $\beta$ -Lactamases in a tertiary care medical centre. J. Clin. Microbiol., 35(8): 2061-2067.
- Ghatole M, Manthalkar P, Kandle S, Yemul V, Jahagirdar V (2004). Correlation of extended spectrum  $\beta$ -lactamases production with Cephalosporin resistance in gram negative bacilli. Indian J. Pathol. Microbiol., 47(1): 82-84.
- Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A (2006). Emerging resistance to carbapenams in a tertiary care hospital in North India. Indian J. Med. Res., 124: 95-98.
- Jabeen K, Zafar A, Hasan R (2005). Frequency and sensitivity pattern of extended spectrum  $\beta$ -Lactamase producing isolates in a tertiary care hospital laboratory of Pakistan. J. Pak. Med. Assoc., 55(10): 436-439.
- Jain A, Roy I, Gupta M K, Kumar M, Agarwal SK (2003). Prevalence of Extended Spectrum  $\beta$ -Lactamases producing gram negative bacterial septicaemic neonates in a tertiary care hospital. J. Med. Microbiol., 52: 421-425.
- Kader AA, Angamuthu K (2005). Extended-spectrum  $\beta$ -Lactamases in urinary isolates of *Escherichia Coli*, *Klebsiella pneumoniae* and other gram-negative bacteria in a hospital in Eastern Province, Saudi Arabia. Saudi Med. J., 26(6): 956-959.
- Kumar MS, Lakshmi V, Rajagopalan R (2006). Occurrence of extended spectrum  $\beta$ -Lactamases among enterobacteriaceae spp. isolated at a tertiary care institute. Ind. J. Med. Microbiol., 24(3): 208-211.
- Luzzano F, Mezzatesta M, Li CM, Perilli M, Stefani S, Amicosante G, Rossolini GM, Toniolo A (2006). Trends in production of extended-spectrum  $\beta$ -Lactamases among enterobacteria of medical interest: Report of the Second Italian Nationwide Survey. J. Clin. Microbiol., 44(5): 1659-1664.
- M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM (2000). Detection of extended-spectrum  $\beta$ -Lactamases in members of the family Enterobacteriaceae: Comparison of the MAST DD test, the double disc and the E-test ESBL. J. Antimicrobiol Chemother., 45: 881-885.
- Padmini SB, Appalaraju B (2004). Extended Spectrum  $\beta$ -Lactamases in urinary isolates of *E.coli* and *Klebsiella pneumoniae* prevalence and susceptibility pattern in a tertiary care hospital. Ind. J. Med. Microbiol., 22(3): 172-174.
- Paterson DL, Bonomo RA (2005). Extended spectrum  $\beta$ -Lactamases: A clinical update. Clin. Microbiol. Rev., 18(4): 657-686.
- Rafay AM, Al-Muharrmi Z, Toki R (2007). Prevalence of extended spectrum  $\beta$ -Lactamases producing isolates over a 1 year period at a University Hospital in Oman. Saudi Med. J., 28(1): 22-27.
- Rodrigues C, Joshi P, Jain SH, Alphonse M, Krishnan RR, Mehta A (2004). Detection of  $\beta$ -Lactamases in Nosocomial gram



- negative clinical isolates. *Indian J. Med. Microbiol.*, 22(4): 247-250.
- Samaha-Kfoury JN, Araj GF (2003). Recent developments in  $\beta$ -Lactamases and extended spectrum  $\beta$ -Lactamases. *B M J* 327(7425): 1209-1213.
- Shan MC, Anantha KA, Jaya Keerthi SR, Kanungo R, Kumar A, Bhattacharya S, Badrinath S (2004). Learning from an outbreak: ESBL- The essential points. *Indian J. Med. Microbiol.*, 22(4): 255-257.
- Tankhiwala SS, Jalgaonkar SV, Ahamad S, Hassani U (2004). Evaluation of extended spectrum  $\beta$ -Lactamase in urinary isolates. *Indian J. Med. Res.*, 120: 553-556.
- Tenover FC, Mohammed MJ, Gorton TS, Dembek ZF (1999). Detection and Reporting of organisms producing extended-spectrum  $\beta$ -Lactamases: Survey of laboratories in Connecticut. *J. Clin. Microbiol.*, 37(12): 4065-4070.
- Vercauteren E, Descheemaeker P, Ieven M, Sanders CC, Goossens H (1997). Comparison of screening methods for detection of extended-spectrum  $\beta$ -Lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella* spp. In a Belgian Teaching Hospital. *J. Clin. Microbiol.*, 35(9): 2191-2197.
- Villanueva FD, Tupasi TE, Abiad HG, Baella BQ, Candano RC (2003). Extended-spectrum  $\beta$ -lactamase production among *Escherichia Coli* and *Klebsiella spp.* at the Makat Medical Centre: Tentative Solutions. *Phil. J. Microbiol. Infect. Dis.*, 32(3): 103-108.
- Yunsong Y, Weilin Z, Yagang C, Xiang Y, Yilin MA (2002). Epidemiological and antibiotic resistant study on extended-spectrum  $\beta$ -lactamase producing *Escherichia Coli* and *Klebsiella pneumoniae* in Zhejiang Province. *Chinese Med. J.*, 115(10): 1479-1482.