



The detection of Extended Spectrum Beta-Lactamases (ESBLs) producing *Escherichia coli* isolated from clinical samples

Rinki R. Yadav and Poonam B. Chauhan*

KBS Commerce & NATARAJ Professional Sciences College, Vapi-396191, Gujarat, India
(Affiliated to Veer Narmad South Gujarat University, Surat)

*Corresponding author: poonambchauhan@gmail.com

Abstract

Urinary tract infections (UTIs) are a stern public health problem and are caused by a wide range of pathogens, but most frequently by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*. High frequency rates and increasing antimicrobial resistance among uropathogens threaten to greatly raise the economic load of these infections. Few *E.coli* organisms are able to produce an enzyme called extended spectrum beta-lactamases (ESBLs) that cause high resistance to all beta-lactam antibiotics with the exception of carbapenems or cephamycins but inhibited by beta-lactam in combination with inhibitors like clavulanic acid, sulbactam and tazobactam. This study was to determine the prevalence of ESBLs. Isolation and identification of ESBLs producing *E.coli* isolation and its confirmation via different confirmative tests. The study included total of 150 clinical specimens of urine and pus. *E.coli* isolates were screened positive was study for ESBL detection by DDST and DDDT method. The ESBL percentage was more in female urine samples.

Keywords: Urinary tract infections, ESBLs, DDST.

1. Introduction

Extended spectrum beta-lactamases are the plasmid encoded beta-lactamases enzymes that are most often derived from mutation in the older beta-lactamases (SHV-1, TEM-1 and TEM-2, CTX-M, OXA-lactamases), in which CTX-M is the most common ESBL type worldwide (Paterson *et al.*, 2005). ESBLs are produced by many gram negative bacteria belongs to the family Enterobacteriaceae, in which *Escherichia coli* and *Klebsiella pneumoniae* are the two most common ESBL producers causing UTIs and nosocomial infections. The older beta-lactamases also called penicillinase confer resistance to beta-lactam antibiotics like penicillin, carbapenems, cephamycin and sensitive to third generation cephalosporins. However ESBLs mediate resistance to extended spectrum cephalosporins (third generation cephalosporins, 3GCs) and monobactams with the exception of cephamycins or carbapenems (Paterson

et al., 2005). Beta-lactam antibiotics inhibit the cell wall synthesis in bacteria, by the covalent attachment to penicillin-binding protein (PBP), which is a peptidoglycan transpeptidase enzyme responsible for catalyzing the final steps in cell wall synthesis and damage of the bacterial cell by hydroxyl radicals (Kohanski *et al.*, 2007). Beta-lactam antibiotics have beta-lactam ring in their structure, Beta-lactamases enzyme carry out destruction of that beta-lactam ring by addition of water molecules. Due to point mutation in older beta-lactamases, ESBLs have serine at their active site and attack the amide bond in the lactam ring of antibiotics causing their hydrolysis (Chaudhary and Aggarwal, 2004). This type of enzymatic destruction is the most common mechanism of resistance to antimicrobial agents accomplish by reduce accumulation of drug in the bacterial cells.

These ESBLs are only inhibited by beta-lactamase inhibitor combination (BLIs) like clavulanic acid, sulbactam and tazobactam, any gram negative bacteria that are resistant to 3GCs but sensitive to beta-lactam inhibitor combination (BL/BLI) are considered as ESBL producing bacteria (Paterson *et al.*, 2005). The materialization of ESBLs producing bacteria especially *Escherichia coli* and *Klebsiella pneumoniae* now resist the additional class of antibiotics therefore, infections particularly UTIs and nosocomial infections which were caused by them become a great therapeutic challenge (Emily *et al.*, 2005). The rapid emergence and irrepressible increase in antimicrobial resistance of pathogenic organisms especially ESBLs producers is widely accepted as a serious problem that has been observed over last decade and now a critical concern for the development of therapeutic drugs against them (Canton *et al.*, 2006; Pitout *et al.*, 2008 and Ramphal *et al.*, 2006).

2. Materials and Methods

2.1 Sample collection and handling

The present study includes analysis of 150 clinical samples (30 Pus and 120 Urine samples), which were collected from Haria L.G Rotary Hospital Lab. and Devanshi Lab., Vapi, Gujarat. All the clinical samples were collected in sterile container and vortexed before proceeding.

2.2 Isolation and identification

For the isolation of *E.coli* all the clinical samples were inoculated first on Mac-conkey's agar media and incubated at 37°C for 24 h. After incubation culture characteristics of isolates were identified based on various biochemical characterizations as per standard microbiological techniques.

2.3 Antibiotic susceptibility testing

E.coli isolates were subjected for antibiotic susceptibility testing by Kirby-Bauer disc diffusion method on Muller-Hinton agar as per the CLSI guidelines. The turbidity of inoculum suspension was adjusted to 0.5 Macfarland's standard. Then this suspension was inoculated onto Muller-Hinton agar plate by lawn culture. After that various antibiotic discs were placed using sterile forceps and pressed gently to confirm proper contact with medium. The plates were then incubated at 37°C for 24 h. The zone of inhibition was measured and interpreted. The antibiotic discs of Nalidixic acid (NA), Piperacillin

(PC), Amikacin (AK), Ciprofloxacin (CIP), Tetracycline (TE), Ofloxacin (ZN), Gentamycin (GEN), Norfloxacin (NX), Ampicillin (AMP), Levofloxacin (LE) and Amoxycylav (AMC) were used for Antibiotic susceptibility test.

2.4 Detection of ESBLs

2.4.1 ESBLs screening test

The isolated organisms were screened for possible ESBL production using Ceftriaxone (CTR), Ceftizoxime (CI), Cefoparazone (CP), Cefixime (SF), Cefotaxime (CTX), Ceftazidime (CAZ) discs. The turbidity of inoculum suspension was adjusted to 0.5 Macfarland's standard. The suspensions were inoculated onto Muller-Hinton agar plate by lawn culture. The above mentioned six discs were placed at a gap of 20 mm each. Later each plate was incubated at 37°C for a period of 24 h. The zone which is formed around the discs were measured and interpreted as sensitive and resistant. The isolates which showed resistance to any one of these antibiotic discs were considered as screen positive. These isolates were further tested for ESBL production by Double disc synergy test (DDST) and Double disc diffusion test (DDDT) method. The other isolates which were sensitive for the same antibiotics were not included in the further tests for ESBL production.

2.4.2 Detection of ESBL by double disc synergy test (DDST)

The isolated organisms which were screened positive for ESBL production were used in DDST. The turbidity of inoculum suspension was adjusted to 0.5 McFarland's standard. This suspension was inoculated onto Muller-Hinton agar plate by lawn culture. The disc containing Amoxycylav (amoxicillin + clavulanic acid) was placed at center of the plate. Ceftriaxone (CTR) and ceftazidime (CAZ) discs were placed with the interdisc distance of 15mm from the combination disc. The plates were incubated at 37°C for 24 h. Enhancement of zone of inhibition towards amoxycylav by any of these antibiotic discs was considered as positive result (ESBL producer).

2.4.3 Double-disc diffusion test (Phenotypic confirmatory test)

The DDDT test was used to confirm that isolates were positive by DDST. Standardized inoculum conforming to 0.5 McFarland standards of suspected ESBL-producing isolates were inoculated as described

before. Then, 4 discs containing Ceftriaxone (CTR), cefoperazone (CP) with and without sulbactam were placed at the recommended distance from each other on the plate. The plates were incubated at 37°C for 24 h. A greater than or equal to a 5 mm increase in the zone diameter for both ceftriaxone and cefoperazone tested in combination with sulbactam (CL and CM respectively) versus its zone diameter when tested alone (CTR and CP respectively) confirmed an ESBL-producer. Any of the cephalosporins antibiotic discs with and without combination can be used for the DDST.

3. Results

3.1 Isolation and identification

A total examination of 150 clinical samples (urine and pus) collected from both outdoor and indoor patients were analyzed, only 65 sample restrain *E.coli* were obtained from the urine (n=50) and from pus samples

(n=15) (Table3.4). Age distribution of patients was between 5- 65 years with a mean of 25-28 years. More isolates were recovered from females (n=40) as compared to male (n=25) which were suffering from symptomatic UTIs. From 65 *E.coli* isolates 20 isolates were from indoor patients and 45 isolates were from outdoor patients.

3.1 Antibiotic susceptibility test

In antibiotic susceptibility test the Antibiograms revealed that 4 (6.15%) isolates were resistant to amikacin, 3 (4.61%) were resistant to amoxycylav, 36 (55.38%) were resistant to ampicillin, 5 (7.69%) were resistant to gentamycin, 8 (12.30%) were resistant to levofloxacin, 35 (53.84%) were resistant to nalidixic acid, 25 (38.46%) were resistant to norfloxacin, 10 (15.38%) isolates were resistant to ofloxacin, 8 (12.30%) isolates were resistant to piperacillin (Table 3.1).

Table 3.1 Antibiotic susceptibility profile of the isolated *E.coli* (n=65)

Sr. No.	Antimicrobial Agents	Resistant Number (%)	Intermediate Number (%)	Susceptible Number (%)
1	Amikacin (AK)	4 (6.15)	17 (26.15)	44 (67.69)
2	Amoxycylav (AMC)	3 (4.61)	22 (33.84)	40 (61.53)
3	Ampicillin (AMP)	36 (55.38)	19 (29.23)	10 (15.38)
4	Gentamycin (GEN)	5 (7.69)	25 (38.46)	35 (53.84)
5	Levofloxacin (LE)	8 (12.30)	15 (23)	42 (64.61)
6	Nalidixic acid (NA)	35 (53.84)	18 (27.69)	12 (18.46)
7	Norfloxacin (NX)	25 (38.46)	20 (30.76)	20 (30.76)
8	Ofloxacin (ZN)	10 (15.38)	5 (7.69)	50 (76.92)
8	Piperacillin (PC)	8 (12.30)	20 (30.76)	37 (56.92)
10	Tetracycline (TE)	9 (13.84)	6 (9.23)	50 (76.92)

3.2 Screening for ESBL producing *E.coli* isolates

In a disc-based ESBL screening tests cefixime, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone (cephalosporins) were used as screening indicators in which 30 (46.15%) isolates of *E.coli* were resistant to cefixime, 30 (46.15%) isolates were resistant to cefoperazone, 29 (44.61%) isolates were resistant to cefotaxime, 30 (46.61%) isolates were

resistant to ceftazidime, 20 (30.67%) isolates were resistant to ceftizoxime and 40 (61.53%) isolates were resistant to ceftriaxone (Table 3.2). Among the six cephalosporins *E.coli* isolates showed maximum resistant to ceftriaxone and ceftazidime which were found to be the best antibiotics for the ESBL phenotypic confirmatory tests when using either with the DDST and DDDT.

Table 3.2 Screening for ESBL producing *E.coli* using screening indicators.

Sr. No.	Antimicrobial agents	Resistant Number (%)	Intermediate Number (%)	Susceptible Number (%)
1	Cefixime (SF)	30 (46.15)	3 (4.61)	25 (38.46)
2	Cefoperazone (CP)	30 (46.15)	19 (29.23)	16 (24.61)
3	Cefotaxime (CTX)	29 (44.61)	21 (32.3)	15 (23)
4	Ceftazidime (CAZ)	35 (53.84)	20 (30.76)	15 (23)
5	Ceftizoxime (CI)	20 (30.76)	15 (23)	30 (46.15)
6	Ceftriaxone (CTR)	40 (61.53)	15 (23)	10 (15.38)

It is found that ESBL producers are more resistant to cephalosporins which were used for screening as

compared to non-ESBL producers that were sensitive for the same cephalosporins (Table 3.3).

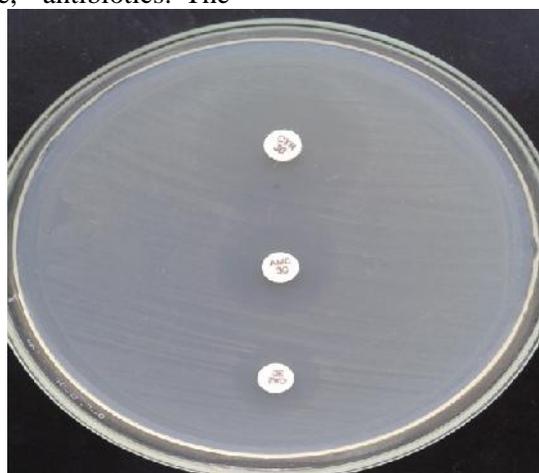
Table 3.3 Comparison of susceptibility to Cephalosporins between ESBL producer (n=13) and non-ESBL producer (n=15) isolates (total isolates n=65).

Sr. No.	Antimicrobial agents	ESBL Producers			Non-ESBL producers		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
1	Cefixime (SF)	10 (15.3)	3 (4.6)	0	0	3 (20)	12 (18.4)
2	Cefoperazone (CP)	11 (16.9)	2 (3)	0	0	2 (3)	13 (20)
3	Cefotaxime (CTX)	12 (18.4)	1 (1.5)	0	0	1(1.5)	14 (21.5)
4	Ceftazidime (CAZ)	12 (18.4)	1 (1.5)	0	0	1 (1.5)	14 (21.5)
5	Ceftizoxime (CI)	10 (15.3)	3 (4.6)	0	0	2 (3)	13 (20)
6	Ceftriaxone (CTR)	13 (20)	0	0	0	1(1.5)	14 (21.5)

3.4 Double disk synergy test (DDST)

Using standard double disk synergy test (DDST) as screening method for identifying potential ESBL producers, ceftriaxone was the most efficient antimicrobial in screening isolates as potential ESBL producers followed by ceftazidime in the present study. In this test, a disc containing amoxicillin-clavulanate (AMC) was placed in proximity to discs containing ceftazidime, ceftriaxone, antibiotics. The

results showed that the clavulanate in the amoxicillin-clavulanate disc diffused through the agar and inhibited the β -lactamase surrounding the ceftriaxone disk. Enhancement of the inhibition zone of any of the third generation cephalosporins test, on the side facing the amoxicillin-clavulanate disc was interpreted as a positive test for ESBL (Fig 3.1). (12.3%) *E.coli* isolates were confirmed as ESBL producers by this double disk synergy test (DDST).

**Fig 3.1 Double disk synergy test (DDST) showing enhancement of zone towards amoxyclav disc by ceftriaxone (CTR).**

3.5 Double disk diffusion test (DDDT)

Double disc diffusion test (DDDT) is a phenotypic confirmatory test for ESBL producing isolates. In this with and without combination of inhibitor antibiotics were used. Those isolates which were produce ESBL

showing zone size of more than 5mm in the disc containing antibiotic and inhibitor as compared to the disc which contain only antibiotic (without any inhibitor). Clavulanic acid was the good inhibitor for ESBL producing isolates (Fig 3.2).

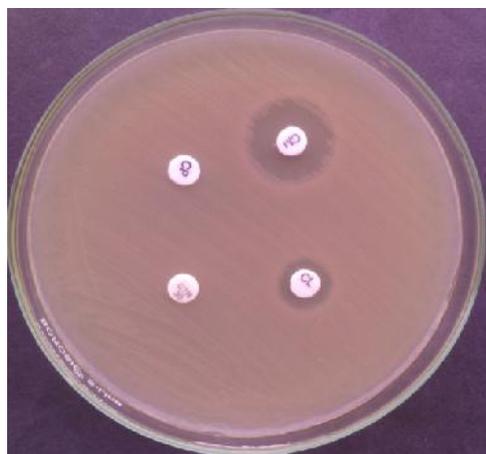


Fig 3.2 Phenotypic confirmatory test, Double disc diffusion test (DDDT) of an ESBL producing isolate showing zone size of more than 5mm in the disc with ceftriaxone/sulbactam (CL) and cefoperazon /sulbactam (CM) (i.e. antibiotics with inhibitor) as compare to ceftriaxone (CTR) and cefoperazone (CP) alone (i.e. antibiotics without inhibitor).

Table 3.4 Results of ESBL producing *E.coli* isolates in urine and pus specimens (n=65)

Sr. No.	Source for isolation	<i>E.coli</i> Isolates No.	<i>E.coli</i> Screened positive No. (%)	ESBL producers No. (%)	Non-ESBL producer No. (%)
1	Urine	50	23 (35.38)	11 (16.92)	12 (18.46)
2	Pus	15	5 (7.69)	2 (3.07)	3 (4.61)
Total		65	28 (43)	13 (20)	15 (23)

Among 150 clinical specimens 65 *E.coli* were isolated from urine (50) and pus (15) in which 28 (43%) isolates were screened positive for ESBL production. Out of which only 13 (20%) isolates were ESBL producers and 15 (23%) isolates were non-ESBL producers (Table 3.3).

4. Discussion

Multi-drug resistance (MDR) is a major problem in the management of uroparhogens (Tankhiwala and Jalgaonkar., 2004; Akram and Shahid., 2007; Hasan and Nair, 2007). The worldwide emergence of Multi-drug resistance (MDR) pathogens is a growing concern which are usually found in those hospitals where antibiotics use is frequent and the patients are in critical conditions (Shahanara Begum *et al.*, 2013). ESBL producing isolates are mostly associated with UTIs which is similar to the present study (Paterson *et al.*, 2005). Therapeutic options for the UTIs which are

caused by the ESBL producers have become increasingly limited (Metri *et al.*, 2011). Study carried out by Naik and Desai, (2012) the more *E.coli* isolates were recovered from female (n=76) as compared to male (n=49), which is in accordance with this findings. The other study carried out by (Ritu Agrawal *et al.*, 2009), the prevalence of *E.coli* was reported 50% among gram negative isolates, a lower incidence, i.e. 43.3% *E.coli* was observed in present study.

The resistance and sensitivity pattern of antibiotic discs which were used in the present study is similar to the study which is carried out by (Naik *et al.*, 2012 and Ndiba *et al.*, 2013). (Shukla *et al.*, 2004) screened the isolates by using cefotaxime (Ce), ceftazidime (Ca), ceftriaxone (Ci) discs and found 88.3% of isolates which were resistant to one of the above mentioned third generation cephalosporins and 72% were resistant to all the three drugs. (Rodrigues *et al.*, 2004) screened the isolates by using aztreonam (Ao),

cefotaxime (Ce), ceftazidime (Ca), ceftriaxone (Ci) and cefpodoxime (Cep) and found that cefpodoxime (Cep) is the most sensitive screening agent. In present study we have used six cephalosporins to screen possible ESBL production. Since there are variations among the ESBLs in their ability to hydrolyse various cephalosporins, it is difficult to pick up any one as the best screening agent. However we observed that ceftriaxone (Ci), ceftazidime (Ca), cefpodoxime (Cep) are the best screening agents. Aztreonam (Ao) too has fared well in demonstrating resistance against these isolates. Use of single or 2 screening agents might sometimes miss the detection of resistant isolates. Hence it was stated that use of three or more screening discs improves the rate of detection. The study carried out by (Harwalkar *et al.*, 2013) compared the resistance pattern to cephalosporins between ESBL producer and non-ESBL producer isolates and found that ESBL producer isolates resist the cephalosporins in larger amount as compared to non-ESBL producer isolates which is similar to the present study.

DDST method is technically simple and inexpensive (Gaurav Dalela *et al.*, 2012). In the study carried out by (Ndiba *et al.*, 2013) detected 90% of 30 ESBL producing isolates by DDST using Cefotaxime (Ce), Ceftriaxone (Ci), and Ceftazidime (Ca) antibiotics which antibiotics are similar to the present study. In the study carried out by Naik and Desai, (2012) 66% of the isolates were found to be ESBL producers when tested with Cefotaxime/Clavulanic acid (Ce/Cec) combination and 63% with Ceftazidime/Clavulanic acid (Ca/Cac) combination. (Tankhiwala *et al.*, 2004) reported 48.3% and (Ritu Aggarwal *et al.*, 2009) reported 40% ESBL producers *E.coli* isolates which were higher than the present study.

This type of similar study i.e. DDDT was also carried out by (Grover *et al.*, 2013) using two antibiotic discs, Ceftazidime (Ca) and Ceftazidime/clavulanic acid (Ca/Cac) and found that ESBL producing isolates showing zone size of more than 5mm in the disk with Ceftazidime/clavulanic acid (Ca/Cac) as compared to Ceftazidime (Ca) which is in accordance with the present study but different antibiotics were used for DDDT in present study i.e. Ceftriaxone (CTR), Cefoperazone (CP) and Ceftriaxone/sulbactam (CL), Cefoperazone/sulbactam (CM). The DDDT also carried out by (Ibrahim *et al.*, 2013) using four antibiotic discs Ceftazidime and cefotaxime with and without clavulanic acid which were different from the present study but their results were similar to the present study, that zone of inhibition of antibiotic disc

with clavulanic acid was more than 5mm as compared to antibiotic disc without clavulanic acid.

5. Conclusion

Present study, the frequency of ESBL producing *E.coli* is more in female urine samples that were suffering from asymptomatic UTIs. ESBL production is the most common mechanism for the spread of multi drug resistance organisms. The prevalence of ESBL producing *E.coli* was high therefore, it is necessary to monitor ESBL production by antimicrobial susceptibility testing to reduce these resistant organisms and routine screening of ESBL should be performed on all isolates showing decreases susceptibility to one or more of third generation cephalosporins. It is also needed to improve the methods used for ESBL detection.

Acknowledgments

I would like to special thanks to my main supervisor Mrs. Poonam B Chauhan (Principal, KBS and NATARAJ College, Vapi, India) for her continuous guidance, suggestions, active cooperation, constant supervision, and support. I would also like to thanks Mr. Mayur Gahlout and Mr. Hiren Prajapati, Miss. Mita vakilwala (Assistant Professor) and non-teaching staff for their inspiring encouragement, constructive criticism and help in carrying out this work successfully. Finally, I would like to thanks to my parents and all my friends.

References

1. Akram, Shahid, M. Etiology and antibiotic resistance patterns of community-acquire urinary tract infections in JNMC Hospital Aligarh, India. *Annals of Clinical Microbiology and Antimicrobials*. (2007);6:4.
2. Anandkumar Harwalkar, Jagadeesh Sataraddi, Soham Gupta, Raksha Yoganand, Achut Rao, Hiresave Srinivasa. The detection of ESBL-producing *E.coli* in patients with symptomatic urinary tract infections using different diffusion methods in a rural setting. *Journal of Infection and Public Health*. (2013);6:108-114.
3. Canton R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, et al. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe, *Jornal of Clinical Microbiology Infectious disease*. (2008);14:144-53.

4. Chaudhary, U, Aggarwal, R, Extended spectrum beta lactamases(ESBL)- An emerging threat to clinical therapeutics, Indian Journal of Medical Microbiology (2004) 22(2):75-80.
5. Col Naveen Grover, Brig A.K Sahni, Col S. Bhattacharya. Therapeutic challenges of ESBLs and AmpC beta-lactamases producers in a tertiary care center. Medical Journal Armed Forces India. (2013):4-10.
6. Emily PH, Adam DL, Theoklis EZ, Irving N, Neil OF, Warren BB, Ebbing L. Risk factors for increasing multidrug resistance among Extended spectrum beta-lactamases producing *E.coli* and *K.pneumoniae*, Journal of clinical infectious disease. (2005);40:1317-1324.
7. Gaurav Dalela. Prevalence of Extended Spectrum Beta-Lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan, Indian, Journal of Clinical Diagnostic. (2012);6(2):182-187.
8. Hasan AS, Nair D. Resistance patterns of urinary isolates in a tertiary Indian hospital. Journal of Ayub. Medical, College, Abbottabad. (2007);19(1):39-41.
9. Mutasim Elhadi Ibrahim. Prevalence of extended spectrum beta-lactamases producing *E.coli* from hospital in Khartoum state, Sudan. Oman Medical journal. (2013).
10. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. Cell (2007) Sep 7;130(5):797-810.
11. Metri Basavaraj C., Jyothi P., and Peerapur Basavaraj V. The Prevalence of ESBL among Enterobacteriaceae in a Tertiary Care Hospital of North Karnataka, India, Journal Clinical Diagnostic. (2011);5(3):470-475.
12. Jigna Naik and Pratibha desai. Antibiotic resistance pattern in urinary isolates of *E.coli* with special reference to extended spectrum beta-lactamases production. International Journal of Pharmacy and Life sciences. (2012);3:1498-1502.
13. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clinical, Microbiology. (2005);18(4):657-86.
14. Patrik Mutharia Ndiba. Phenotypic detection and susceptibility pattern for the detection of extended spectrum beta-lactamases producing *K.pneumoniae* isolates in Nairobi, Kenya. Open Journal of Microbiology. (2013);3:91-94.
15. Pitout JD, Nordman P, Laupland KB, Poirel I. Emergence of Enterobacteriaceae producing extended spectrum beta-lactamases (ESBLs), Clinical Microbiology Chemotherapy. (2008);56:52-59.
16. Ramphal R. Extended spectrum Beta-lactamases and clinical outcomes: current date, Journal of Clinical Infectious Disease. (2008);42:164-172.
17. Ritu Aggrawal, Uma Chaudhry, Rama Sikka, Rohtak. Detection of Extended Spectrum -lactamase Production Among Uropathogens, Journal of laboratory physicians. (2009);(1):7-10.
18. Rodrigues C, Joshi SH, Alphone M, Radhakrishnan, R, Mehta A. Detection of beta-lactamases in nosocomial gram negative clinical isolates. Indian Journal of Medical Microbiology. (2004);22:247-250.
19. Shahanara Begum, Md Abdus Salam, Kh Faisal Alam, Nurjahan Begum, Pervez Hassan and Jalaluddin Ashrafu Haq. Detection of extended spectrum -lactamase in *Pseudomonas* spp. isolated from two tertiary care hospitals in Bangladesh. Biomedcentral. (2013);6:7.
20. Shukla I, Tiwari R, Agrawal M. prevalence of extended spectrum-lactamases producing *K.pneumoniae* in a tertiary care hospital, Indian Journal of Medical Microbiology. (2004);22:87-91.
21. Tankhiwale SS, Jalgaonkar SV. Evaluation of extended spectrum beta-lactamases in urinary isolates, Indian Journal of Medicine. (2004); 120(6):1005-8.

Access this Article in Online	
	Website: www.ijarbs.com
	Subject: Medical Microbiology
Quick Response Code	

How to cite this article:

Rinki R. Yadav and Poonam B. Chauhan. (2016). The detection of Extended Spectrum Beta-Lactamases (ESBLs) producing *Escherichia coli* isolated from clinical samples. Int. J. Adv. Res. Biol. Sci. 3(5): 9-15.