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ANTIBACTERIAL SUSCEPTIBILITY PROFILE OF *ESCHERICHIA COLI* IN A PRIVATE HOSPITAL, INDIA

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ABSTRACT

E.coli is a freely available gram negative bacteria among the natural resources like gut of animals which at the same time emerges to be one of the most causative organisms for stomach and urine related infections in humans. The presence of Extended-spectrum beta-lactamase ESBL type of drug resistance is constantly evolving and are under dynamic flux posing a global threat to public health programs. Also, there is a significant necessity for regular antimicrobial sensitivity surveillance not only for the presence and spread of ESBL genes but also for both urban and rural populations for more informed and structured treatment.At an institutional level, practices that can minimize the spread of such organisms include clinical and bacteriological surveillance of patients admitted to intensive care units and antibiotic cycling, as well as policies of restriction, especially on the empirical use of broad-spectrum antimicrobial agents. The present study focuses on the trends of antibacterial susceptibility and resistance among clinically isolated *E.coli* for rational prescribing. Susceptibility and resistance data of *E.coli* were collected from a tertiary care hospital's Microbiology Department over a period of three years. The data collected included patient's source (ICU or ward), specimen of the isolate, antibacterial susceptibility and resistance profile. *E.coli* was identified from the sample on the basis of colony and microscopic morphology. Commercially available antibiotic disks were used for antimicrobial susceptibility testing as per Kirby–Bauer disk diffusion method and Clinical Laboratories Standard Institute CLSI guidelines.The pattern of antibiotics used within prescribing pattern for E.coli were found out to be 33.3%, 30.3% and 27.5% in the study period. The identification of EBSL, AmpC, Carbapenamase were carried out as they were identified to be as resistant strains and more complicated in terms of identification and treatment.Susceptibility to third generation Cephalosporins, Gentamycin, Imipenem,Meropenem, Amikacin showed a narrow increase in resistance level against *E. coli*.

KEYWORDS: *Escherichia Coli, ESBL, AmpC, Carbapenemase, Cephalosporins and Meropenem*

INTRODUCTION

Escherichia coli (*E.coli*) is the most prevalent facultative anaerobic bacteria in the gastrointestinal tract of humans and animals. It is usually a harmless microbe, also causing a number of significant illnesses[1.] The discovery of *E.coli* as an 'emerging pathogen' was made in the same year when the association of sporadic cases of Hemolytic Uremic Syndrome (HUS) with Cytotoxin - producing fecal *E. coli* was been observed[2]. World Health Organization (WHO) grades *E.coli* as one of the major agents of concern associated both with hospital and community acquired infections[3]. *E.coli* has come into existence in several countries as a cause of prevalent bloody and non-bloody diarrhea, HUS and Thrombotic Thrombocytopenic Purpura. This can lead to fatal complications that occur approximately at a rate of 5-10% of all cases [4,5] . The emergence of Extended-spectrum β-lactamases (ESBL) type of resistance offered by *E.coli* is supported by many reports[6]. This is a major threat to the already hostile community of physician in the hospitals who are looking for alternative and novel antibiotics to tackle ESBL type of infections[7]. ESBLs are a rapidly evolving group of β-lactamases which share

the ability to hydrolyze third-generation Cephalosporins and Aztreonam but are inhibited by Clavulanic acid. They represent the first example in which β-lactamase - mediated resistance to β-lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes[8]. Identifying ESBL-producing organisms is a major challenge for the clinical microbiology laboratory. Multiple factors contribute to this, including production of multiple different β-lactamase types by a single bacterial isolate[9]. At an institutional level, practices that can minimize the spread of such organisms include clinical and bacteriological surveillance of patients admitted to intensive care units and antibiotic cycling; as well as policies of restriction, especially on the empirical use of broad-spectrum antimicrobial agents such as the third and fourth-generation Cephalosporins and Quinolones[10-12] The present study focuses on the trends of antibacterial susceptibility and resistance among clinically isolated *E.coli*. The data gathered is anticipated for rational prescribing.

MATERIALS AND METHODS

Susceptibility and resistance data of *E.coli* were collected from a tertiary care hospital's Microbiology Department over a period of three years (calendar year 2012, 2013 and 2014) in a prospectively designed data collection form. The data collected includes patient's source (ICU or ward), specimen of the isolate, antibacterial susceptibility and resistance profile. *E.coli* was identified from the sample on the basis of colony, microscopic morphology and biochemical reactions[13]. Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines[14]. Commercially available antibiotic disks were used for antimicrobial susceptibility testing. The antibiotic disks used are Ampicillin (10 µg), Piperacillin (100 µg), Piperacillin / Tazobactam(100/10µg), Amoxicillin / Clavulanic acid (20/10µg), Cefoperazone/Sulbactam (75/10 µg), Ceftazidime/Clavulanate (30/10 µg), Cefoperazone (75 µg), Cefoxitin (30 µg), Ceftazidime (30µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Aztreonam (30 µg), Imipenem(10 µg), Amikacin (30 µg), Gentamycin (10 µg), Ciprofloxacin (30 µg), Ofloxacin (5 µg),Norfloxacin (10 µg), and Nitrofurantoin (300 µg). The quality control of antibiotic sensitivity was done using *E.coli* ATCC 25922 and *E.coli* ATCC 35218 (for β-lactam/β-lactamase inhibit or combination).

RESULTS

Specimen wise distribution of *E. coli* is given in Table – 1. Prevalence of *E. coli* among the positive cultures is found to be 33.3%, 30.3% and 27.5% in 2012, 2013 and 2014 respectively. This shows that the pattern of antibiotics used was well versed within the prescribing pattern for *E.coli*.

Table 1
Specimen wise distribution of E.coli

Specimen		2012		2013		2014	
		Total Number of samples with positive culture	Number of *E.coli* isolates	Total Number of samples with positive culture	Number of *E.coli* isolates	Total Number of samples with positive culture	Number of *E.coli* isolates
Urine	Ward	1247	724	1332	714	336	169
	ICU	190	48	249	83	105	28
Pus/EENT Swabs/Stool/BF	Ward	614	110	574	89	237	58
	ICU	117	17	110	12	45	7
Respiratory	Ward	224	14	301	17	106	13
	ICU	300	19	420	18	205	18
Blood	Ward	301	72	238	34	90	30
	ICU	191	58	175	46	77	8
Total no. of *E.coli*		**1062**		**1013**		**331**	

The identification of EBSL, AmpC, Carbapenemase were carried out as they were identified to be resistant strains and are more complicated in terms of identification and treatment. The EBSL strain substantially increased from 52.5 % to 72.3 % in the three years of study. The AmpC and Carbapenemase strains showed a significant drift in 2013 but tend to increase in 2014 as shown in Table – 2. This suggests choosing the right antibiotics is required to minimize the increase of these strains.

Table 2
Distribution of E.coli and resistant strains

	2012				2013				2014			
	Total *E.coli* isolates	EBSL (%)	AMPC (%)	CARBAPENAMASE (%)	Total *E.coli*	EBSL (%)	AMPC (%)	CARBAPENAMASE (%)	Total *E.coli*	EBSL (%)	AMPC (%)	CARBAPENAMASE (%)
Ward	920	61	12	8	386	65	1	3	51	78	4	1.8
ICU	142	44	12	5	110	63	1	8	51	66.6	5.8	2
Total		52.5	12	6.5	507	64	1	5.5	107	72.3	4.9	1.9

The susceptible profiles (in terms of % of isolates) of *E.coli* over three year period is given in Table – 3. Susceptibility to third generation Cephalosporins (Figure-1), Gentamycin (Figure-3), Imipenem, Meropenem (Figure-5), Amikacin (Figure-4) showed a narrow increase in resistance level against *E. coli*. The Fourth generation Cephalosporin, Cefepime(Figure-2), showed a slight decrease in resistance when compared to 2012 and 2014. Even though, antibiotics like Colistin, Piperacillin/Tazobactam were lately introduced, it remains to be more susceptible to the gram negative bacteria

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Table 3
Susceptible profile (in %) of E.coli

Year / Antibacterial	2012			2013			2014		
	Ward (N = 920)	ICU (N = 142)	Average	Ward (N = 386)	ICU (N =110)	Average	Ward (N=51)	ICU (N = 51)	Average
FLUOROQUINOLONES	37	19	28	21	4	12.5	-------	-------	-------
3RD GEN CEPHALOSPORINS	36	15	25.5	21	4	12.5	16	25	20.5
CEFIPIME	36	19	22.5	28	20	24	16	25	20.5
GENTAMYCIN	48	40	44	44	48	46	40	57	48.5
AMIKACIN	93	63	78	29	29	29	96	98	97
CEFOPERAZONE/SULBACTAM	83	75	79	85	82	83.5	87	88	87.5
IMIPENAM	95	92	93.5	----------	---------	--------	98	98	98
MEROPENEM	95	92	93.5	55	48	51.5	98	98	98
AMOXYCILLIN	-------	--------	--------	8	9	8.5	10	12	16
AMOXYCILLIN/CLAVULANATE	-------	--------	--------	32	46	39	40	55	47.5
PIPERACILLIN/TAZOBACTAM	-------	--------	--------	---------	----------	--------	71	75	73
COLISTIN	-------	--------	--------	---------	----------	--------	100	100	100
CO-TRIMAXAZOLE	-------	--------	--------	1	4	2.5	0.7	2	1.35

DISCUSSION

The *E.coli* is a freely available gram negative bacteria among the natural resources. It is also found in the cattle and human gut flora. At the same time, it emerges to be one of the most causative organism for stomach and urine related infections in both children and adults. The rise of EBSL has become a growing threat in the clinics to identify and treat the resistant strains.A three year study was carried out to identify the antibacterial strains prominent in the ICU and wards. The study showed a major increase in the EBSL strains in comparison to AmpC and Carbapenemase strains. This can worsen the disease condition in the patients infected and can also be fatal. The increase in prevalence needs to be addressed at the earliest through creating awareness and sensitizing the prescribers regarding the threat of ESBL strains.The study conducted by Kibret *et.al* showed an increased number of positive cultures in urine sample which

was similar to our study[15]. The gram negative bacteria are prone to cause urinary tract infection and thus are found widely in urine samples. In another study taken up by Ugwu *et.al* found that in stool samples EBSL strains were mostly present among the *E.coli* strains. In the present study, the EBSL strains were mostly present along with AmpC and Carbapenemase which was not identified in the Ugwu *et.al* study[16]. The susceptibility of different antibiotics was tested in the following years and the susceptibility profile is graphically presented below. The new generation antibiotics showed high resistance in comparison to previously prescribed antibiotics. The prescribing pattern needs to be altered to come across the resistant strains.

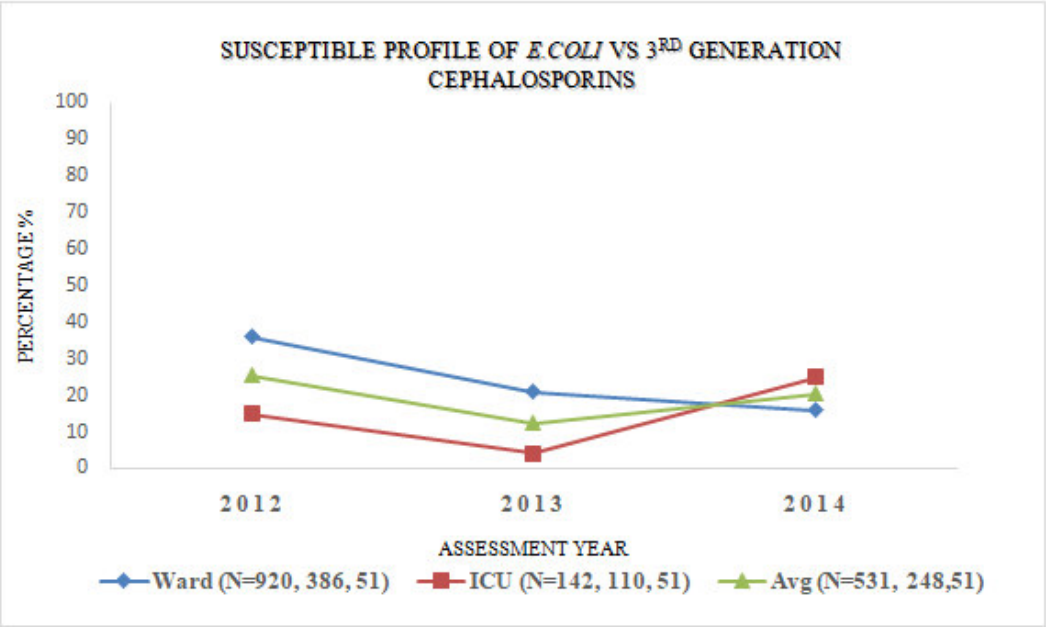

Figure 1
Susceptible profile of E.coli vs 3rd generation Cephalosporins

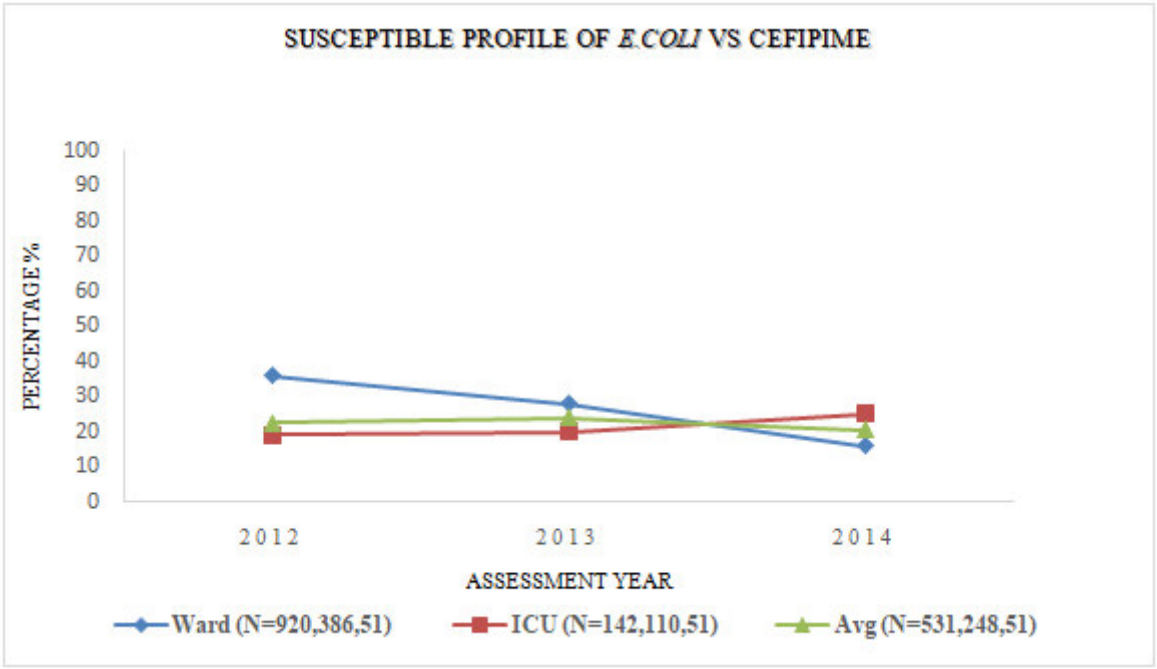

Figure 2
Susceptible profile of E.coli vs Cefipime

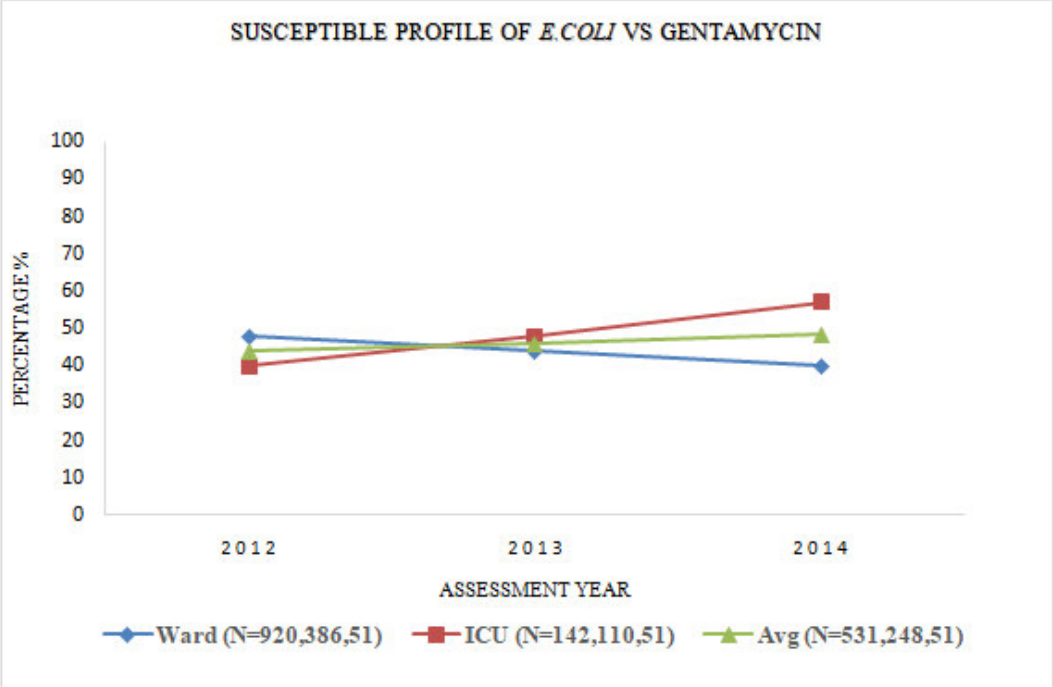

Figure 3
Susceptible profile of E.coli vs Gentamycin

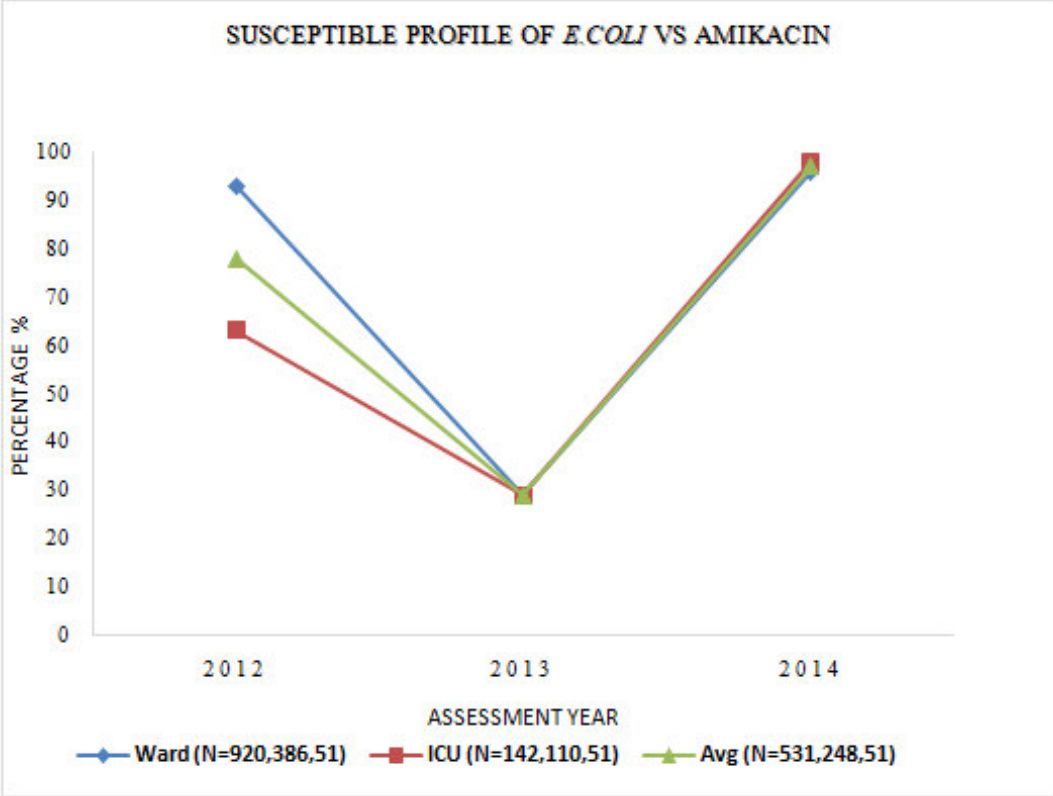

Figure 4
Susceptible profile of E.coli vs Amikacin

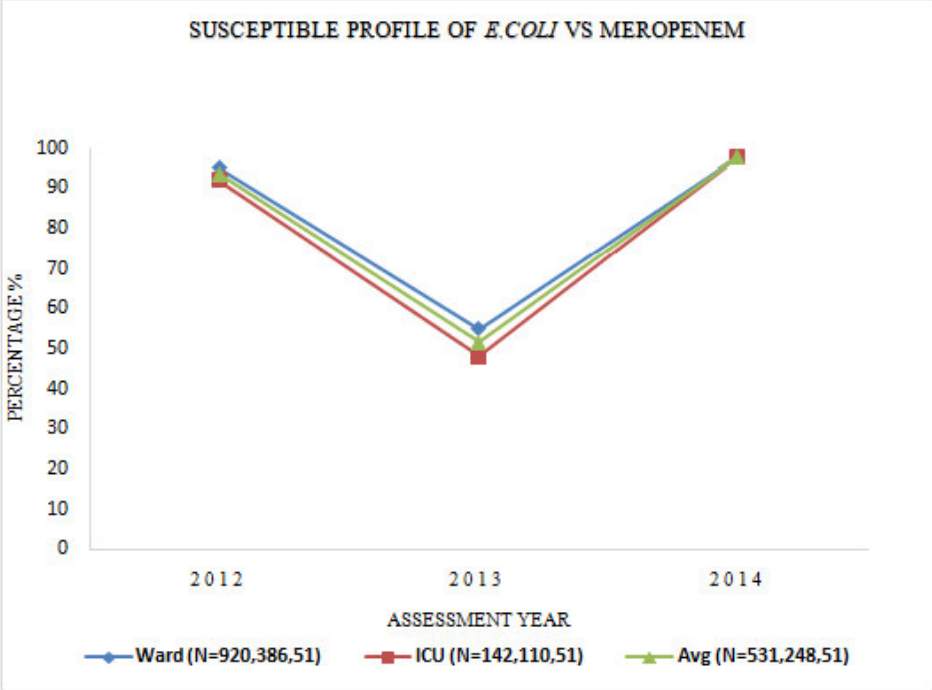

Figure 5
Susceptible profile of E.coli* vs *Meropenem

CONCLUSION

The presence of ESBL type of drug resistance is constantly evolving and is under dynamic flux. Hence, it poses a global threat to public health programs. Again , there is a significant necessity for regular antimicrobial sensitivity surveillance not only for the presence and spread of ESBL genes but also for urban and rural populations for more informed and structured treatment.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, *et al*. Health care associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Annals of internal medicine. 2002 Nov 19;137(10):791-7.
2. Karmali M, Petric M, Steele B, Lim C. Sporadic cases of haemolytic-uraemic syndrome associated with faecal Cytotoxin and Cytotoxin-producing *Escherichia coli* in stools. The Lancet. 1983 Mar 19; 321(8325):619-20.
3. Griffin, P.M., Tauxe, R.V. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli* and the associated hemolytic uremic syndrome. Epidemiol. Rev. 1991 Jan 1;13,60-98.
4. World Health Organization. Antimicrobial resistance: global report on surveillance. World Health Organization; 2014.
5. Wachsmuth, I.K. Summary: public health; epidemiology; food safety; laboratory diagnosis, in: Karmali, M.A., Goglio, A.G. (Eds), Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections. Amsterdam, Elsevier Science BV, 1994;pp. 35.
6. Patricia A.Bradford. Extended-Spectrum β-Lactamases in the 21st Century: Characterization, Epidemiology and Detection of This Important Resistance Threat. Clin Microbiol Rev 2001 Oct 1; 14(4): 933-951
7. Jacoby GA, Munoz-Price LS. The new beta-lactamases. N Engl J Med 2005; 352(4):380-91.
8. Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. Antimicrob Agents Chemother.1989 Aug; 33(8):1131–6.
9. Rawat D, Nair D. Extended-spectrum β-lactamases in Gram Negative Bacteria. J. global infectious diseases. 2010 Sep;2(3):263.

10. Bhattacharya S. ESBL-From petri dish to the patient. Indian J Med Microbiol. 2006 Jan 1;24(1):20–4.
11. Samaha-Kfoury JN, Araj GF. Recent developments in β lactamases and extended spectrum β lactamases. BMJ. 2003 Nov 20;327(7425):1209–13.
12. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clin Microbiol Rev. 2005 Oct 1;18(4):657–86.
13. Akhilesh Kushwaha, Vishal Kumar Singh, Juhi Bhartariya , Priya Singh and Khadeeja Yasmeen. Isolation and identification of E. coli bacteria for the synthesis of silver nanoparticles: Characterization of the particles and study of antibacterial activity. Euro. J. Exp. Bio. 2015;5(1):65-70
14. Clinical Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Clinical Laboratory Standard Institute; Wayne, Pennsylvania, USA: 2012; Vol. 32; pp. 70–71.
15. Kibret M, Abera B. Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. African health sciences. 2011;11(3):40-5
16. Ugwu MC, Edeani GI, Ejikeugwu CP, Okezie U, Ejiofor SO. Antibiotic Susceptibility Profile of *Escherichia coli* and *Salmonella* Causing Childhood Diarrhoea in Awka Municipality, South-eastern Nigeria. Clin Microbiol. 2017; 6(277):2.