Section A-Research paper

Determination of antimicrobial resistance patterns and detection of ESBL and AMPC: Producing uropathogenic *E. coli*

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Background: Uropathogenic E. coli's multidrug resistance has become a potential threat to global health. The purpose of the current study is to evaluate the antimicrobial against Extended Spectrum -Lactamases (ESBL) and AmpC producer *E. coli* isolates.

Methodology: All clinical isolates of *Escherichia coli*, isolated from UTI patients were evaluated for susceptibility to a panel of antimicrobials and were analysed for the ESBL and AmpC using Vitek 2.

Results and conclusions: *Total 1911 no of urine samples were screened out of these 782 were having the significant bacteriuria. In this 226 sample have shown the growth of E.Coli.* Maximum no of patients are of age group more than 60 years (35.41%).52.21% were ESBL producers. The AST of ESBL *E.Coli* showed isolates were sensitive to Tigecycline (100%) and were 99.15% sensitive to Imipenem and Meropenem followed by 97.46% to Amikacin. The 37 no wee identified the AmpC. Further research is required to detect ESBL and AmpC enzyme production simultaneously in such isolates. It is necessary to detect ESBL and AmpC-producing E. coli early to prevent treatment failure. Continuous surveillance and prudent antibiotic use, as well as the implementation of effective infection control measures, are essential.

Keywords: Uropathogenic E.Coli, ESBL, AmpC, MDR

Antimicrobials are often used in hospitals, with up to 70% of patients in the intensive care unit (ICU) and 35% of adult ward patients, respectively, receiving one on any given day[1]. The patient is at danger of getting multidrug resistant (MDR) pathogens due to this heavy antibiotic burden. Exogenous crosscontamination, or the transmission of MDR organisms from other patients or the healthcare environment, or selection pressure exerted on the patient's own microbiome, which gives mutant strains a competitive edge[2].

ESBLs are resistant to all beta-lactams with the exception of cephamycins and carbapenems. Therapeutic failure has been described with third-generation cephalosporin with an in vitro pattern of intermediate or greater sensitivity, thus once ESBLs production is confirmed, the strain must be considered resistant to all beta-lactams, except for carbapenems and cephamycins. However, cefoxitin, cefotetan and cefamandole are not recommended as treatment options due to the risk of developing antibiotic resistance during the course of the treatment[3]**.**

Carbapenems are the treatment of choice for severe infections, since they seem to be the only ones capable of maintaining bactericidal activity for 24 hours against high inocula, with the exception of ertapenem. In case of septic shock, meropenem and imipenem are mostly recommended. In mild cases, piperacillin/tazobactam and amoxicillin/ clavulanic acid or fluoroquinolones may be considered[4]. ESBLs-producing E. coli is the most frequently isolated infectious agent in this group.

Since its discovery in 1983, Extended Spectrum -Lactamases (ESBL)-producing organisms have become a significant issue in the field of infectious diseases[5].Since they can totally hydrolyze oximino-beta lactams like third generation cephalosporins, which are typically used to treat hospital acquired infections, the ESBL and AmpC generating E. coli have posed a serious hazard to hospitalized patients. This plasmid-mediated resistance may spread more easily due to the use of broad-spectrum oral antibiotics and likely subpar infection control procedures. Ambulatory patients with chronic illnesses constitute another patient population that may host ESBL-producing microbes in addition to identified populations at risk[5-9]. On the chromosomes of several Enterobacteriaceae and a few other organisms are encoded the therapeutically significant cephalosporins known as AmpC -lactamases. Where they have a role in the resistance to most penicillins, cephalothin, cefazolin, cefoxitin, and -lactamase inhibitor/-lactam combos. Clavulanic acid, an ESBL inhibitor, had no effect on the activity of AmpC -lactamase. While they were given Group 1 in Bush et al functional categorization system, AmpC enzymes are classified as Class-C in the Ambler structural classification of beta lactamases. Members of the Enterobacteriaceae family can have either chromosomal or plasmid-mediated AmpC -lactamase genes. AmpC overproduction and outer membrane porin mutations can lessen sensitivity to carbapenems, especially in plasmid-mediated AmpC producers[10-12].

Urinary tract infection is one of the most common infections, mostly caused by gram negative bacteria and out of which E Coli is most common isolate. E coli isolates are becoming

more ESBL & AmpC Beta lactamase producing Uropathogens. The present study was undertaken to study antibiotic resistance patterns, including ESBLs & Amp C beta lactamases in urinary isolates of E Coli.

Material and methods

After approval of Institutional Ethics committee the present study was conducted at a tertiary care hospital. A total of 1911clean catch midstream urine samples were collected in a sterile container for the period Jan 2022 to Oct 2022. Urine samples were transported immediately to Department of Microbiology for processing. Then the urine samples were inoculated on MacConkey's and Blood agar plates by using calibrated loop delivering 0.001 ml of sample and incubated at 37°C aerobically for 24 hrs. For gram negative bacilli more than 10^5 colonies per ml of single organism were considered significant.

All isolates were identified and tested for susceptibility by the Vitek 2 system (bioMerieux; Inc., Durham, NC, USA) using the Gram-negative strain cards AST N280, AST N281, AST N405, AST N406. The isolates were tested for susceptibility for the following antimicrobial agents: amikacin, cefepime, cefoperozone, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, piperacillin/tazobactam, tigecycline, and trimethoprim sulfamethoxazole.

All results were interpreted using the Advanced Expert System (software version VT2-R04.03). Using the Vitek 2 system according to the manufacturer's recommendations. Whilst the Vitek 2 Advanced Expert System^{TM} (AES), which automatically compares minimum inhibitory concentrations (MICs) of antibiotics to a large database and suggests possible mechanisms of resistance, tends to favour the most conservative options to ensure safe patient treatment, it may thereby add to the over-calling of ESBLs. An isolate was considered an ESBL producer if the phenotypic interpretation by the AES of the Vitek 2 included ESBL with or without decreased outer membrane permeability and not an ESBL if only wild type or β-lactamases other than ESBLs were suggested by AES.

Results

Summarization and analysis of data was carried out by using software statistical package for social sciences (SPSS-20 version). Data was condensed in the form of tables. Statistics like percentages and mean were computed. Chi square test was applied to study the association. Chi square test were said to be significant when probability was less than 0.05.

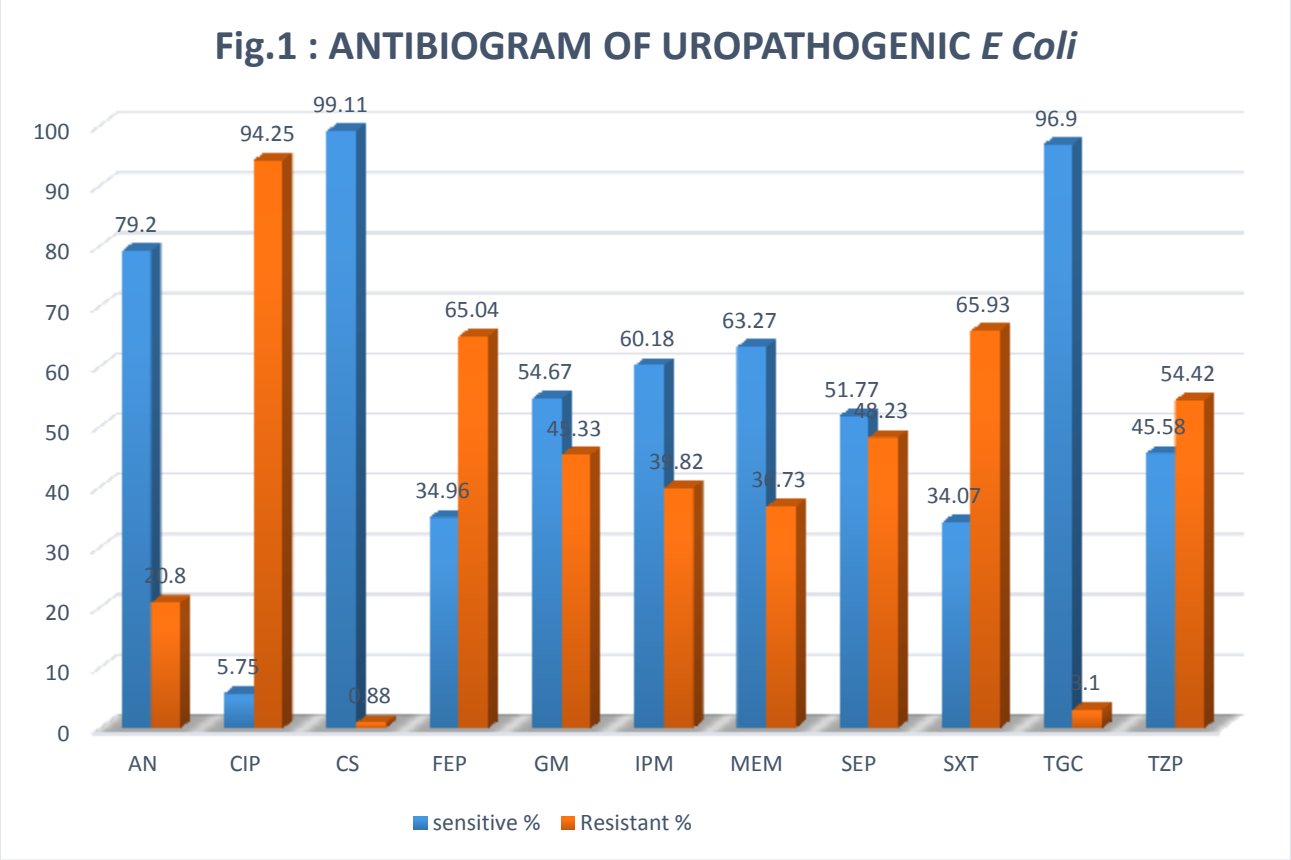
The Department of microbiology received total 1911 no of urine samples during Jan. 2022 to Oct. 2022. Out of these total urine samples, 782 no of sample were positive for one or two organisms having the significant bacteriuria. In this 226 sample have shown the growth of *E.Coli*.

Table1: Age and Sex distribution of Cases.

Sr. NO.	Age Group (Yrs.)	Male (%)	Female (%)	Total (%)
1	<20	6(5.22)	8(7.21)	14(6.19)
2	21 – 40	32(2.83)	33(29.73)	65(28.76)
3	41 – 60	28(24.35)	39(35.14)	67(29.64)
4	>60	49(42.60)	31(27.92)	80(35.41)
5	**Total**	**115(100)**	**111(100)**	**226(100)**

$\chi^2 = 6.088$, df = 3, P = 0.1074

The age and sex distribution of UTI cases having growth of *E.Coli* are shown in table1. Maximum no of patients are of age group more than 60 years (35.41%) followed by age group of 41to 60, followed by 21to 40 age group. Maximum number of males are of age group more than 60 years while maximum females are of age group 41to 60 years.



AN-AMIKACIN
CIP-CIPROFLOXIN
CS- COLOSTIN
FEP- CEFEPIME
GM- GENTAMICIN
IPM- IMIPENEM

MEP- MEROPENEM
SEP-CEPEROZONE
SXT- TRIMETHOPRIM SULFAMETHOXAZOLE
TGC- TIGECYCLINE
TZP- PIPERACILLIN/TAZOBACTUM

Table2: Antimicrobial susceptibility testing pattern

Antibiotic	ESBL Positive(118)			ESBL Negative(108)		
	Sensitive (%)	Resistant (%)	Intermediate (%)	Sensitive (%)	Resistant (%)	Intermediate (%)
Amikacin	115 (97.46)	3(2.54)	0 (00.00)	64(59.26)	44(40.74)	2(1.85)
Ciprofloxacin	8 (6.78)	101(85.59)	9(7.63)	5(4.63)	103(95.37)	3(2.78)
Colistin	118 (100)	0(0.00)	0(00.00)	106(98.15)	2(01.85)	0(00.00)
cefepime	61(51.69)	57(48.31)	0(00.00)	19(17.59)	89(82.41)	0(00.00)
Gentamicin	78 (66.10)	40(33.90)	0(00.00)	45(41.67)	63(58.33)	1(0.93)
Imipenem	117 (99.15)	1(0.85)	1(00.85)	20(18.52)	85(78.70)	13(12.04)
Meropenem	117 (99.15)	0(0.00)	6(05.08)	26(24.07)	82(75.93)	0(00.00)
Cefoperozone	99 (83.90)	13(11.02)	0(00.00)	18(16.67)	90(83.33)	1(0.93)
Trimethoprim/ sulfamethoxazole	48 (40.68)	70(59.32)	0(00.00)	29(26.85)	79(73.15)	0(00.00)
Tigecycline	118 (100)	0(0.00)	0(00.00)	101(93.52)	7(06.48)	6(5.56)
Piperacillin/tazobactum	87 (73.73)	25(21.19)	6(05.08)	17(15.74)	91(84.26)	0(00.00)

Fig. 1 & Table No 2 shows the Antimicrobial Susceptibility testing pattern in all E.coli isolates. There were 118 ESBL positive *E.Coli*. The ESBL positive *E.Coli* isolates all the isolates were sensitive to Tigecycline (100%) and were 99.15% sensitive to Imipenem and Meropenem followed by 97.46% to Amikacin. for Cefoperozone 83.90% sensitivity while for Piperacillin/Tozobactum 73.73% isolates were sensitive. ESBL *E.coli* showed 51.61% & 40.68% sensitivity to Cefepime and Trimethoprim/Sulfamethoxazole respectively.

The out of 226 *E.Coli* grown in the UTI cases, the AES identified the 118 samples as the extended spectrum B lactamases. Out of the remaining 108 *E.Coli*, the Carbapenemase Resistant Enterobacteriaceae (CRE) stains were 84. The difference between *E.Coli* and ESBL & CRE E.Coli is statistically significant (Table No. 3)

Table No. 3: ESBL producing *E.Coli.*

Organism	Non ESBL	ESBL	Total
E.Coli	24	118	142
E.Coli CRE	84	00	84
Total	108	118	226

$\chi^2 = 146.069$, df = 1, P = < 0.005

Table No. 4: AmpC producing *E.Coli*

Organism	Non AmpC	AmpC	Total
E.Coli	105	37	142
***E.Coli* CRE**	84	00	84
Total	189	37	226

$\chi^2 = 20.192$, df = 1, P = < 0.005

Table 4 shows the distribution of AmpC producing *E.Coli*. The 37 no of UTI cases have identified the AmpC out of 226 *E.Coli*. The AmpC *E.Coli* & *E.Coli* CRE, the difference is statistically significant.

The maximum number of ESBL *E.Coli* were found in the age group of more than 65 years (45 nos.) followed by 33 nos. in age group of 41 to 60 years and 32 isolates of ESBL *E.Coli* in the age group of 21 to 40 years and very less no (08) isolates in the age group of below 20 years (Table5). The sex wise distribution of ESBL *E.Coli* is equally distributed there are 59 females and 59 in males.

Table 5: Age distribution of ESBL & non ESBL Cases.

Sr. NO.	Age Group (Yrs.)	Non ESBL	ESBL	Total (%)
1	<20	6	8	14
2	21 – 40	33	32	65
3	41 – 60	34	33	67
4	>60	35	45	80
5	**Total**	**108**	**118**	**226**

Discussion

The prevalence of ESBL-producing Enterobacteriaceae has risen alarmingly in the current period of expanding usage of broad-spectrum antibiotics[13]. ESBLs and AmpC -lactamase producing *E. coli* are currently a global problem for hospitalized patients. The prevalence of ESBLs and AmpC production among urine isolates varies substantially globally, geographically, and through time, and is subject to fast change.

In the present study in 226 urine sample showed *E.Coli* growth. The ESBL production was observed in 52.21% of *E. Coli*. The similar results 50-80 % ESBL production have been reported in other studies also[14-18]. Antimicrobial selection varies from region to region, and so does ESBL production.

In present study 37 isolate were detected as AmpC producing E.Coli out of total 226 E.Coli (16.37%).Bakshi R[6] et al showed the 66.6% isolates by AmpC disc method. In the same study they have reported Susceptibility of ESBLs and AmpC producers E. coli to fosfomycin,

imipenem, nitrofurantoin and amikacin were found to be 100%, 98%, 83.8% and 72% respectively[18]. In the present study our ESBL isolates were Imipenem & Meropenem 99% sensitive, Amikacin 97.46% Piperacillin/Tozobactum 73.73% sensitive to these antibiotics. Similarly other studies have also reported susptibility of ESBL producers to imipenem and amikacin at 100% & 68 % respectively[9, 19].

In present study out of 226 *E.Coli*, the AES identified the 118 samples as the extended spectrum B lactamases. Out of the remaining 108 *E.Coli*, the Carbapenemase Resistant Enterobacteriaceae (CRE) stains were 84(77.78%) which is also the alarming sign for treating the patients in tertiary care hospital. The increasing drug resistance of bacteria is the major cause of treatment failure of urinary tract infection cases in hospitals.

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