

Prevalence of Extended Spectrum Beta Lactamase (ESBL) production in Escherichia coli bacteria isolates from patients suffering from urinary tract Infections.

Imran Ahmad Malik

Ph.D. Scholar, Centre for interdisciplinary Biomedical Research,
NIMS University Jaipur Rajasthan

Abstract: Urinary tract infections (UTI) are caused by presence and growth of microorganism in urinary tract. Bacteria are primary organisms that cause UTI. Among all, gram-negative bacteria are predominant and amount for 80-85% of infection and E. coli is most common organism causing UTI.

Objectives:

1. To isolate and identify Escherichia coli (E. coli) from urine samples.
2. To perform antimicrobial testing of isolated pathogens.
3. To detect ESBL production in cephalosporin resistant isolates of E. coli.

Material & Methods: The study was carried out in 2022. A total 1,675 samples were received in Bacteriology section from various OPD and IPD of AIMS hospital. E. Coli was identified on the basis of colony characteristics, gram staining morphology and biochemical tests. Antimicrobial susceptibility testing was done by Vitek2 system. For ESBL Detection Appropriate antibiotic discs were placed on the Muller Hinton agar media and plates were incubated at 37°C for 18-24 hrs. Results were interpreted in accordance with Central Laboratory Standards Institute (CLSI) guidelines.

Results

Total of 1675 Urine Samples were processed, 650 (38.80%) yield significant growth and 1025(61.20%) samples showed no growth. Out of total positive samples 112 samples were reported as positive for E. coli. More number of E. coli isolates was obtained from males (55.0%) than in female (45.0%). Age group mostly affected was 51-60 years (21.42%) followed by 61-70 years (20.53%). most of the isolates were found to be highly sensitive to Colistin (87%), Nitrofurantoin (82%), Meropenem (79%), Tigecycline (79%). They showed resistance towards Ciprofloxacin (95%), Ampicillin (91%), Cefuroxime (91%), Cefepime (79%), Ceftriaxone (77%), Nalidixic Acid (77%) and 71% to AmoxicillinClavulanic Acid.

Conclusion

The present study highlights that ESBL positive isolates of E. coli are prevalent not only to the hospitalized patients but also in the community.

Keywords Escherichia coli, Urinary tract infection, antimicrobial susceptibility testing, gram-negative bacill.

Date of Submission: 14-01-2023

Date of Acceptance: 30-01-2023

I. INTRODUCTION

Urinary tract infection refers to a wide range of clinical disorders, from asymptomatic bacteria in the urine to severe kidney infection with sepsis as a result. [1] Each year, millions of people get infected with various forms of bacterial infections, but urinary tract infection is one of the most prevalent. In comparison to community residents, hospitalized patients are more likely to get a urinary tract infection. Because of poor hygiene, lifestyle, malnutrition, and environmental circumstances, the disease is more prevalent in poorer countries.[2] Urinary tract infection (UTI) is an inflammatory condition of the urinary tract caused by bacteria that have grown abnormally in the urinary system. A urinary tract infection can cause short-term morbidity such as fever, dysuria, and lower abdomen pain, as well as irreversible kidney scarring. [3] Urinary tract infections are mainly caused by gram negative bacteria which account for 80–85% and the leading causative organisms are Escherichia coli (E. coli) (75.5–87% of UTI cases) followed by Klebsiella species, Citrobacter, Acinetobacter, Enterobacter, Providencia, Pseudomonas, Serratia and Proteus species. [1] Antibiotic resistance of bacteria is commonly seen in daily medical practice with multidrug-resistant Gram-negative bacteria posing the greatest threat to human health. Beta-Lactam antibiotics are the commonly prescribed antibiotics to treat bacterial infections. However, most gram-negative bacteria produce beta-lactamases enzymes which are their major defense mechanism against beta-lactam antibiotic [4] Beta Lactam antibiotics which are commonly use to treat bacterial infections which include penicillin, cephalosporins, Carbapenems, and monobactams.[2] Extended-

Spectrum Beta-lactamases (ESBLs) are plasmid-mediated beta-lactamases recognized for their ability to hydrolyze 3rd- and 4th-generation cephalosporins (oxyiminocephalosporins) and monobactams but not cephamycin or Carbapenems. Additionally, these enzymes are repressed by beta-lactamase inhibitors as clavulanic acid and tazobactam.^[5] ESBLs are encoded by transferable conjugative plasmids which often code resistant determinants to other antibiotics. The plasmid-mediated resistance against cephalosporins can spread among related and unrelated gram-negative bacteria. ESBLs are mostly the products of point mutations at the active site of TEM and SHV enzymes. . To isolate and identify *Escherichia coli* (*E. coli*) from urine samples. To perform antimicrobial testing of isolated pathogens. To detect ESBL production in cephalosporin resistant isolates of *E. coli*.

II. MATERIALS AND METHODS

A study was carried out for six months from October 2021 to March 2022. Urine samples were received from patients suspected of having Urinary tract infection, OPD and IPD (admitted in Adesh hospital Bathinda in various departments). They, All the patient's identification details like Hospital CR. Number, Lab. Number, patient name, age, gender & Department name with specimen collection date were recorded on a formatted proforma. A total no of 1,675 patients' samples were received, during the study period in various departments from all age groups

Urine specimens were inoculated by semi-quantitative technique on blood agar, and MacConkey's agar. Plates were incubated at 37°C for overnight the colony-forming unit >10⁵ CFU/ml were considering significant. The isolates were thus identified by colony characteristics gram staining and were subjected to biochemical reactions.^[6]

Identification of the bacterial Isolates

Urine specimens were inoculated by semi-quantitative technique on blood agar, and MacConkey's agar. Plates were incubated at 37° C overnight the colony-forming unit >10⁵ CFU/ml were considering significant. The isolates were thus identified by colony characteristics gram staining and were subjected to biochemical reactions. (IMVIC +++-)^[6]

Antimicrobial sensitivity testing

Antimicrobial sensitivity testing of *E. coli* isolates was performed by Vitek2 system using GNB AST Card recommended by Clinical and laboratory Standards Institute (CLSI) guidelines. An isolated colony of *E. coli* was picked and added sterile saline solution provided by the manufactures BioMerieux to make a suspension equivalent to a 0.5 McFarland standard, adjusted by using a Densi CHEK plus (BioMerieux) and further processed as per the manufacture's instruction AST panel AST-N280 was used for *E. coli*.^[6]

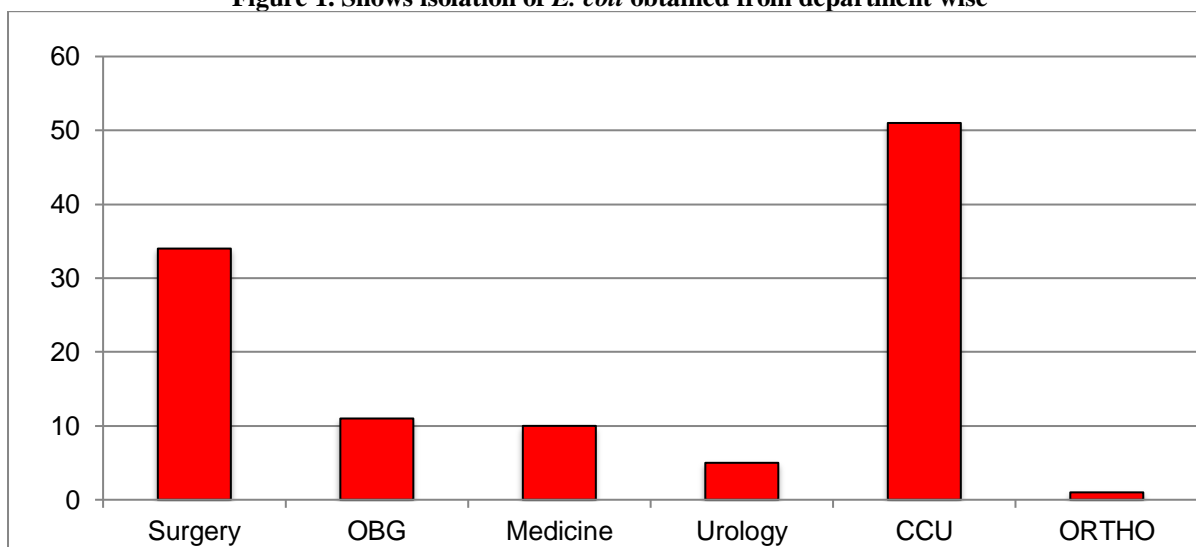
III. Results

Out of total 1675 Urine samples processed; 650 samples showed growth, culture positivity was 38.80% and 1025 were culture negative. Out of 650 samples to obtain 112 *E. coli* isolates, (55.0%) samples were received from males and (45.0%) from female patients. Maximum isolates were obtained from patients with age group of 51-60 years (21.42%) as compared to other age groups. followed by 61-70 years (20.53%). (Table 1)

Table 1 shows age wise distribution of *E. coli*:

Age (In years)	NO. OF ISOLATES	PERCENTAGE
<20	06	5.35
21-30	08	7.14
31-40	15	13.39
41-50	17	15.17
51-60	24	21.42
61-70	23	20.53
>71	19	16.96
Total	112	100

Figure 1. Shows isolation of *E. coli* obtained from department wise



Antimicrobial Sensitivity Pattern of *E. coli* Isolates

In our study *E. coli* isolates were found to be highly sensitive to colistin (87%), less to Nitrofurantoin (82%), Meropenem (78%), Tigecycline (78%), Imipenem (75%), amikacin (73%), Ertapenem (68%) Cotrimoxazole (61%), Cefoperazone (57%) and maximum resistance towards Ciprofloxacin (95%), followed by Ampicillin (91%), Cefuroxime (91%), Cefepime (78%), Ceftriaxone (77%), Nalidixic Acid (77%), Clavulanic Acid (71%), Ertapenem (64%) and 50% to Tazobactam.

Antimicrobial agents	No. of sensitivity isolates	No. of resistant isolates
AMPICILLIN	10(9%)	102(91%)
AMOXICILLIN/CLAVULANIC ACID	32(29%)	80(71%)
AMIKACIN	82(73%)	30(27%)
GENTAMICIN	64(48%)	48(52%)
CIPROFLOXACIN	05(5%)	107(95%)
NALIDIXIC ACID	26(23%)	86(77%)
NITROFURANTONIN	92(82%)	20(18%)
CEFUROXIME	10(9%)	102(91%)
CEFTIAXONE	25(22%)	87(78%)
CEFEPIME	24(21%)	88(79%)
CEFOPERAZONE/SULBACTAM	64(57%)	48(43%)
TAZOBACTAM/PIERACILLIN	56(50%)	56(50%)
ERTAPENEM	65(58%)	47(42%)
IMIPENEM	84(75%)	28(25%)
MEROPENEM	88(79%)	24(21%)
TIGECYCLINE	88(79%)	24(21%)
COLISTIN	98(88%)	14(12%)
COTRIMOXAZOLE	68(60%)	44(40%)

Table 2 shows antimicrobial sensitivity pattern of *E. coli* isolates

ESBL production in *E. coli* isolates

Out of 112 *E. coli* 31 were obtained ESBL positive and 81 were obtained ESBL negative.

Table 7 shows ESBL production in *E. coli*.

Total	ESBL Production			
	ESBL Positive	%	ESBL Negative	%
112	31	28%	81	72%

IV. Discussion

In the present study, a total of 1675 urine samples were processed; 650 (38.80%) were cultured positive, and 1025 (61.19%) were cultured negative. The prevalence of UTI due to *E.coli* in the study was found to be 112/650[17.23%].

A study by Lata et al (2015), at InfeXnLaboratorypvt.Ltd, Vishakhapatnam, India, reported 732 urine samples, 161 isolates were recovered positive for *E. coli* with 21.85% prevalence.^[7] Ramesh et al (2017), at Institute of Medical Sciences and Research, Angamaly, Kerala, reported that out of 1000 urine samples 395 isolates were reported positive for *E coli* with 39.5% prevalence.^[8] Prevalence reported by above studies is also slightly higher as compared to present study.

A study by Odongo et al (2020), College of veterinary medicine, Animal Resources and Biosecurity Makerere University, Uganda, reported that out of 100 urine samples 10 isolates showed positive growth of *E. coli* with 10.00% prevalence.^[9] The study by Nirnanjan et al, Odongo et al and Lata et al correlated similarity to the present study. A study by Giri et al (2020), at Universal Collage of Medical Science, Bhairahawa, Nepal. Reported that out of 84 urine samples 45 isolates were reported positive for *E. coli* with 53.00% prevalence.^[10] Prevalence reported by this study is much higher as compared to present study.

In the present study, Urinary tract infection was observed more in males (54%) than in females (46%) mainly due to more admission rate of males as compared to females in the hospital.

Mohammad et al (2016), reported that out of 160 isolates positive for *E. coli* and maximum positive 60% were from females and 40% were males.^[11]

Alqasim et al (2018), reported in his study out of 100 *E. coli* isolates 76% were from females and 24% were from males.^[11] Prevalence reported by these studies is higher in females as compared to males, which is contrary to present study.

A study by Shah et al (2019) reported that out of 1237 isolates were positive for *E. coli* and maximum positive 56.35% were males and 43.65% were females.^[12] Najmi et al (2019), reported in their study that out of total 182 *E. coli* were isolates, maximum were from males 60% and females 40%.^[13] The result reported by Shah et al and Najmi et al are similar to present study.

In the present study, Urinary tract infection was observed more in males (54%) than in females (46%) mainly due to more admission rate of males as compared to females in the hospital.

Mohammad et al (2016) reported that out of 160 isolates positive for *E.coli* and maximum positive 60% were from females and 40% were males.^[11] Alqasim et al (2018), reported in his study out of 100 *E. coli* isolates 76% were from females and 24% were from males.^[11] Prevalence reported by these studies is higher in females as compared to males, which is contrary to present study.

A study by Shah et al (2019) reported that out of 1237 isolates were positive for *E. coli* and maximum positive 56.35% were males and 43.65% were females.^[12] Najmi et al (2019), reported in their study that out of total 182 *E. coli* were isolates, maximum were from males 60% and females 40%.^[13] The result reported by Shah et al and Najmi et al are similar to present study.

E. Coli isolates were found to be highly sensitive to colistin (87%), less to Nitrofurantoin (82%), Meropenem (78%), Tigecycline (78%), Imipenem (75%), amikacin (73%), Ertapenem (68%), Cotrimoxazole (61%), Cefoperazone (57%) and maximum resistance towards Ciprofloxacin (95%), followed by Ampicillin (91%), Cefuroxime (91%), Cefepime (78%), Ceftriaxone (77%), Nalidixic Acid (77%), Amoxicillin-Clavulanic Acid (71%), Ertapenem (64%) and 50% to Piperacillin - tazobactam. A study by Shrestha et al (2016), reported that 84% *E. coli* isolates were resistant to Gentamicin 84%, followed by 72.5% Amikacin, Ceftriaxone 68.55% and Nitrofurantoin (64.5%).^[14] Alqasim et al (2018), reported that 91% *E. coli* were resistant to Ampicillin followed by amoxicillin-clavulanic acid (55%), Gentamicin (12%), Ceftazidime, Cefoxitin, tetracycline and Trimethoprim-sulfamethoxazole 29%,13%,49%, 54%, respectively.^[11] Antibiotics resistance pattern of *E. coli* in present study coincides with that of Alqasim et al. Giri et al (2020), reported that *E. coli* was most sensitive to Nitrofurantoin 43(95%), Ciprofloxacin 41 (91%), and Amikacin 88.8%.^[10]

Antibiotic sensitivity pattern of *E. coli* in present study coincides with that of Giri et al. A study by Sadeghi et al (2022), reported *E. coli* were highly resistant to Ampicillin 83.7%, followed by Amoxicillin/Clavulanic acid 58.6%, Ceftriaxone 56.3% and Ceftazidime 55.1%.^[15]

In the present study out of 112 *E. coli* isolates 31(28%) were ESBL positive.

Andrew et al (2017), reported that out of 67 *E. coli* isolates 65.67% were ESBL positive.^[4] Abayneh et al (2018), reported out of 63 *E. coli* isolates 20.63% were ESBL positive.^[16] Sahu et al (2019), perform study and reported that out of 111 *E. coli* isolates 53.15% were ESBL producers.^[17] Agrawal et al (2019), did study on *E. coli* and reported that out of 181 *E. coli* isolates 30% were ESBL positive.^[18] The study by Agarwal et al and Abayneh et al shows similarity with present study. A study by Haussana et al (2020) reported out of 134 *E. coli* isolates 60% were ESBL positive.^[19] The result of the study by Haussana et al, Sahu et al and Andrew et al shows non-similarity with the present study.

V. Conclusion

In the present study Prevalence of ESBL production was seen in *E. coli*, out of 112 *E. coli* isolates 31 (28%) were ESBL positive. The present study highlights that ESBL positive isolates of *E. coli* are prevalent not only to the hospitalized patients but also in the community. The results obtained emphasizes the emergence of multidrug resistant *E. coli* which is an alarming for clinicians and suggests to change in the treatment options depending upon the antimicrobial susceptibility testing. Hospital treating infectious disease can benefit by integrating antimicrobial stewardship program to combat the emergence of AMR and ESBL.

References

- [1]. Mohammad MA, Alnour TMS, Shakurfo OM, Aburass MM. Prevalence and antimicrobial resistance pattern of bacterial strains isolated from patients with urinary tract infection in Messalata Central Hospital, Libya. *Asian Pacific Journal of Tropical Medicine*. 2016;9(8): 771-776.
- [2]. Shrestha A, Manandhar S, Pokharel P, Panthl P, Chaudhary DK. "Prevalence of Extended Spectrum Beta-Lactamase (ESBL) Producing Multidrug Resistance Gram-Negative Isolates Causing Urinary Tract Infection". *EC Microbiology* 4.5(2016);749-755.
- [3]. Aliero A, Tibyangye J, Maniga JN, Wampande E, Kato CD, Agwu E, and Bazira J. Prevalence of Bacterial urinary tract infections and associated factors among patients attending Hospitals in Bushenyi District Uganda. *International Journal of Microbiology* 2019;1-5(4):24-30.
- [4]. Andrew B, Kagirita A, and Bazira J. Prevalence of Extended Spectrum Beta Lactamase producing microorganism in Patients Admitted at KRRH, Southwestern Uganda. *International Journal of Microbiology*, 2017;1-5.
- [5]. Hassuna NA, Khalralla AS, Farhat EM, Hammad AM and Fattah MA. Molecular characterization of Extended Spectrum Beta Lactamase producing *E. coli* recovered from community - acquired Urinary tract infection in Upper Egypt 2020;2772
- [6]. Collee JG, Marr W. Specimen collection, culture containers and media. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors, Mackie and McCartney practical medical microbiology. 14th ed. Toronto: Elsevier Canada. 2008;1414-27
- [7]. Lata B Galate, RonalBangde. Urinary Tract Infection. Study of Microbiological Profile and its Antibiotic Susceptibility Pattern. *International Journal of current Microbiology and Applied Sciences* 2015; 4(0): 502-7.
- [8]. Kulkarni SR, Peearapur Bv, Sailesh KS. Isolation and Antibiotics Susceptibility pattern of *Escherichia coli* from UTI's infections in a tertiary care hospital of North Eastern Karnataka. *J Nat Sc Biol Med* 2017; 8:176-80
- [9]. Isaac Odongo, Ronald Semambo, Joseph M.Kungu, "Prevalence of *Escherichia. Coli* and Its Antimicrobial Susceptibility Profiles among Patients with UTI at Mulago Hospital, Kampala, Uganda", *Interdisciplinary Perspectives, on Infectious Diseases*, vol. 2020, ArticleID 8042540, 5 pages, 2020. <https://doi.org/10.1155/2020/8042540>.
- [10]. Giri A, Kafle R, Singh GK, Niraula N. Prevalence of *Escherichia coli* in Urinary Tract Infections of children Aged 1-15 Years in a Medical College of Eastern Nepal. *J Nepal Med Assoc* 2020;58(221):11-14.
- [11]. Alqasim A, Jaffal AA, Alyousef A A. Prevalence of Multidrug Resistance and Extended-Spectrum β - Lactamase Carriage of Clinical Uropathogenic *Escherichia coli* Isolates in Riyadh, Saudi Arabia. *Hindawi International Journal of Microbiology* 2018; 12. Shah K. A Comparative Study of Antibiotic Sensitivity Pattern of *Escherichia coli* Isolated from various clinical samples over a period of 3 years. *Int. J. Curr. Microbiol. App. Sci* 2019;8(10): 1243-1249.
- [13]. Najmi A, Karimi F, Kunhikatta V, Varma M, Nair S. Resistance Trend, Antibiotic Utilization and Mortality in Patients with *E. coli* Bacteraemia. *Open Access Maced J Med Sci*. 2019 Apr 15; 7(7): 1119-1123.
- [14]. Anil Shrestha, et al. "Prevalence of Extended Spectrum Beta-Lactamase (ESBL) Producing Multidrug Resistance Gram-Negative Isolates Causing Urinary Tract Infection". *EC Microbiology* 4.5(2016): 749-755
- [15]. Sadeghi M, Saraie SE, Mojtahedi A. Prevalence of ESBL and AmpC genes in *E. coli* isolates from Urinary Tract Infections in the north of Iran. *New Microbes and new infections* 2022; 45:1-6.
- [16]. Abayneh M, Tesfaw G, Abdissa A. Isolation of Extended Spectrum Beta Lactamase producing. *Canadian journal of infection Disease and medical Microbiology*. 2018;1-8.
- [17]. Sahu RK, Pradhan S, Sao S, Murthy R, Agrawal E, Patel A, Panday N. Isolation and identification of various Extended Spectrum Beta Lactamase producing 2017 6 uropathogens in a tertiary care hospital Chhattisgarh. *International Journal of Research in Pharmaceutical Sciences* 2019; 10(4): 3244-3250.
- [18]. Kauffman CA. Diagnosis and management of fungal urinary tract infection. *Infect. Dis. Clin. North. Am.* 2014, 28, 61-74.
- [19]. Hassuna NA, Khalralla AS, Farhat EM, Hammad AM and Fattah MA. Molecular characterization of Extended Spectrum Beta Lactamase producing *E. coli* recovered from community - acquired Urinary tract infection in Upper Egypt 2020; 2772.