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

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***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 AIMSR 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^{o}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 AmoxycillinClavulanic 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.*

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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]BetaLactam antibiotics which are commonly use to treat

bacterial infections which include penicillin, cephalosporins, Carbapenems, and monobactams.[2] Extended-Spectrum Beta-lactamases (ESBLs) are plasmidmediated 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 variousdepartments 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

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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]

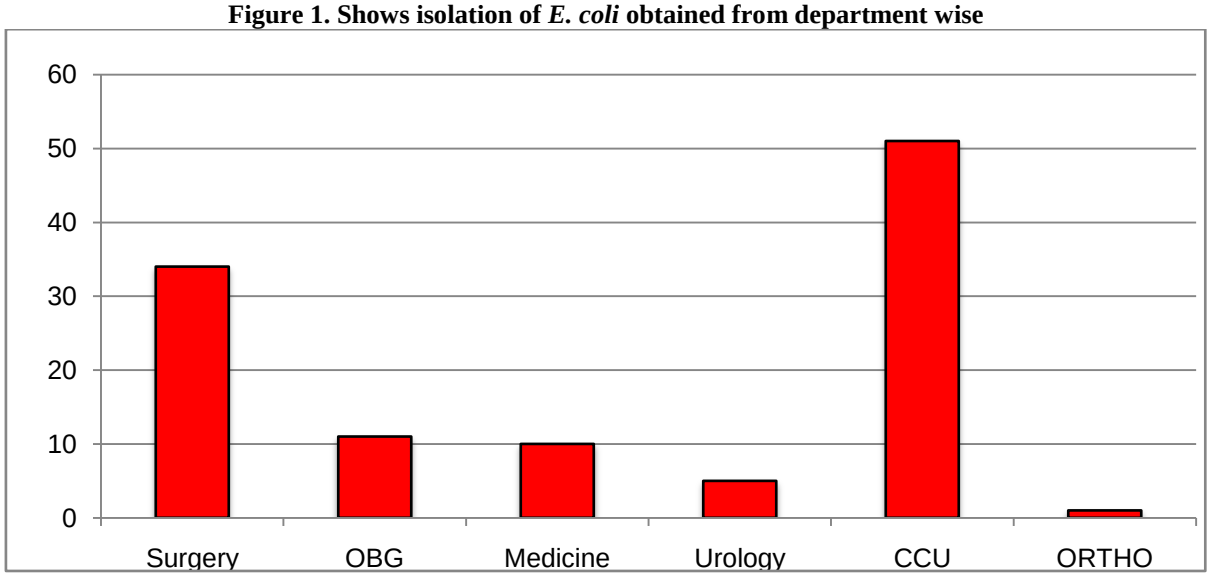
III. Results

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%)
AM0XICILLIN/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/SULBACTUM	64(57%)	48(43%)
TAZOBACTUM/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**			
112	**ESBL Positive**	**%**	**ESBL Negative**	**%**
	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 Niranjan 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. [1]

Alqasim et al (2018), reported in his study out of 100 *E. coli* isolates 76% were from females and 24% were from females. [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.

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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.

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