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A study on evaluation of Escherichia coli isolates in urinary tract infection and its antibiogram in view of Emerging drug resistance at a tertiary care hospital

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Introduction: Urinary tract infection is a common problem in routine clinical practices despite age and sex, and it is also the most common nosocomial infection. The most common uropathogen is Escherichia coli worldwide. **Material and methods:** A retrospective study was conducted from January 2020 to December 2020 in a tertiary-care hospital in South India. All positive urinary cultures with significant bacteriuria received from Inpatient and outpatient wards of various departments were included in the study. The prevalence percentage of different bacteria was studied. Multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) Escherichia coli in different baseline and demographic characteristics were evaluated. **Results:** A total of 2144 urine samples were processed for culture and sensitivity, out of which 531 samples were found to be culture-positive with significant bacteuria. Among 531 samples, 202 (38%) culture positives were due to Escherichia coli infection. Women (62%) were more susceptible to infection. The predominant age group affected among women was 21-30 yrs and men 51-60 years. Of these, 28% exhibited an MDR pattern, 18% XDR and 1.9% PDR. **Conclusion:** Treatment of Urinary tract infection due to E. coli may be difficult due to high resistance to commonly used antibiotics. So continuous surveillance of drug-resistant organisms and evaluation of drug resistance patterns are needed to prevent treatment failure and to reduce the economic burden due to these resistant isolates.

Keywords: Escherichia coli, Multidrug resistance, Community-acquired, Hospital-acquired

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Introduction

Urinary Tract Infection (UTIs) remains one of the most common infections occurring worldwide in routine clinical practice at any time in an individual's life. It may affect the lower (bladder and urethra) and upper urinary tract (kidney and ureter) [1-2]. The clinical presentation ranges from asymptomatic urinary tract infection to severe infection like sepsis leading to death. They are often affected by microbes like bacteria, fungus, and viruses. UTI caused by bacterial infection is predominant compared to the other two microbial infections. Bacteria commonly associated with UTI are Gram Negative organisms like *Escherichia coli, Klebsiella species, Pseudomonas species, and Proteus s*pecies and gram-positive like *Enterococcus species, Staphylococcus aureus, and Staphylococcus saprophyticus.* Among these organisms, *E. coli* accounts for the most common organism causing both community-acquired (CA-UTI) as well as hospital-acquired (HA-UTI) UTI [3].

"Nosocomial or hospital-acquired UTIs are important because of their high prevalence and financial effect in hospitalized patients due to prolonged hospital stays and inappropriate antibiotic use." Community-acquired urinary tract infections (CA-UTIs) account for a large proportion of infectious diseases. The treatment of CA-UTI is mainly based on an empirical regimen without checking for the laboratory report contributing to significant resistance due to frequent and repeated misuse of antibiotics. Urinary tract infection (UTI) is common among men and women, but women show higher incidence compared to men due to their biological conditions [4-5].

Several studies conducted worldwide have shown the emergence of antibiotic-resistant uropathogenic bacteria. They are resistant to commonly prescribed antibiotics leading to reduced therapeutic efficacy and increased demand for novel drugs. The reason for the emergence of multidrug-resistant organisms is repeated exposure to the same antibiotics, widespread use, and cross-transmission of the antibiotic-resistant genes from one bacterium to the other one causing the organism to be resistant to different antibiotic families; finally leading to the evolution of multidrug-resistant organisms [6]. Antimicrobial resistance exhibiting microorganisms can be classified into Multidrug-resistant (MDR), Extensively drug-resistant (XDR), and Pandrug-resistant (PDR) as per ECDC and CDC criteria [7].

In India, many studies have been done on antimicrobial resistance patterns in E. coli isolated from urine which showed high rates of resistance among *E coli* [8]. The present study aims to determine the UTI-causing organisms and determine the prevalence of uropathogenic *E coli* and its antimicrobial resistance pattern.

Material and Methods

This retrospective study was conducted in the Department of Microbiology in a tertiary care hospital over one year from January 2019 to December 2019. Total 2144 urine samples were received to the microbiology department for culture and sensitivity from males and females of all age groups and all the department**s.**

Ethical clearance: After receiving approval from the institutional ethical committee, the study was carried out. The data regarding the urine culture and sensitivity pattern were obtained from the Microbiology laboratory registers.

Collection and Processing of urine sample: Clean catch midstream urine specimen was collected from all patients into a wide-mouthed sterile screw-capped container. Urine samples were taken to a bacteriology lab and processed right away. After receiving to the lab, each specimen was subjected to culture by the semi-quantitative standard loop technique using a 4mm internal diameter loop which collects 0.01 ml of urine sample inoculated on Hichrome UTI agar and MacConkey agar. The plates were incubated aerobically at 370 C overnight. According to Kass criteria, a number ≥ 105 colony forming units (CFU)/mL of urine was considered as significant bacteuria and colony number < 105 CFU/mL was considered as insignificant bacteuria and correlated with clinicians for the report [9].

Isolation and Identification: The organisms isolated from urine culture were identified by standard methods like Growth on Hichrome agar and MacConkey agar, appearance in Grams staining and Biochemical reactions [9].

Antibiotic susceptibility testing: The antibiotic sensitivity test was done on Mueller-Hinton agar by the Kirby-Bauer disc diffusion method. Plates were incubated at 37°C overnight, and the inhibition zone was examined as per the recommendations of the Clinical and Laboratory Standards Institute 2019

(CLSI) [10]. The isolates were tested for (i) β-lactam, which included penicillins like ampicillin (10 µg), cephalosporins like cefuroxime (30 µg), ceftriaxone (30 µg), cefepime (30 µg) and carbapenems like Imipenem (10 µg) and Meropenem (10 µg) (ii) β-lactamase inhibitors like amoxicillin/clavulanate (10/20 µg), ampicillin/ sulbactam (10/10 µg) and piperacillin/tazobactam (100/10 µg); (iii) Fluoroquinolones which included ciprofloxacin (30 µg), Norfloxacin (10 µg) (iv) Aminoglycosides which consists of amikacin (30 µg), gentamycin (10 µg), and netilmicin (30 µg). Besides these four significant classes, a few other antibiotics, nitrofurantoin (300 µg), colistin (10 µg), cotrimoxazole (25 µg), and Minocycline (30 µg) which are also used to treat UTIs, they were included in the current study's antibiotic susceptibility analysis. An isolate was considered MDR, XDR and PDR based on the standardized international terminology proposed by CDC and the European Centre for Disease Prevention and Control (ECDC) standardized international language [7]**.**

Statistical Analysis: Percentages were calculated. The data were analyzed using Chi-square (χ2) test for P-value. Statistical significance was defined when the p-value was <0.05.

Results

In this retrospective study, a total of 2144 urine samples from suspected urinary tract infection patients were received for culture and sensitivity from Inpatient and outpatient of the various department to the microbiology lab. Out of the total urine samples received, 531 samples were culture positive with significant bacteuria, and the remaining are either non-significant bacteuria or sterile. Escherichia coli being the most common pathogen in urine was included for testing its antibiotic profile and prevalence. Escherichia coli was identified by microscopic and biochemical characteristics (Table 1).

Out of 531 uropathogens isolated, Escherichia coli was the most common pathogen, representing 202 (38%) of all the isolates, followed by Enterococcus 140(26%). The least common bacteria isolated is Citrobacter 0.4 %, followed by Morganella 0.6% (Table: 2).

Patient Demographics: During the period of significant study, the difference is observed in the percentage

Table 1: Microscopic and biochemical characteristics of Escherichia coli isolates collected from urinary tract infection patients.

Gram staining	Gram-negative pink-coloured rod-shaped bacteria
Growth on Hichrome & MacConkey Agar	Dark pink colour and lactose fermenting colonies on Mac
Indole test	Positive (Pink colour ring formation)
Simmons citrate test	Citrate is not utilized
Methyl red/Voges-Proskauer test	Positive (appearance of red colour in broth)/negative (Indicated by lack of colour change)
Urease test	Urea is not hydrolyzed

Table 2: Proportion of the bacterial pathogens isolated from the urinary sample

Organism	No of isolates	Percentage (%)
E. coli	202	38%
Enterococcus spp	140	26%
Klebsiella species	94	18%
Pseudomonas species	14	2.5%
Proteus species	13	2.5%
Morganella species	05	0.6%
Staphylococcus species	47	9%
Citrobacter species	02	0.4%
Acinetobacter	14	3%
Total	531	100%

Table 3: Distribution of E coli isolates according to age, gender and source of infection from January 2020 to December 2020.

Age group	Total 202	Males 65 (32%)	Female 137 (68%)	*p 0.00001*	Hospital acquired 98 (49%)	Community acquired 104 (51%)	*p 0.550
0-1Y	18 (9%)	11(17%)	7 (5%)	0.005*	13 (13%)	5 (5%)	0.06*
2-10 Y	16 (8%)	7 (11%)	11(8%)	0.523	7 (7%)	9 (9%)	0.694
11-20 Y	14 (7%)	5 (7.5%)	8 (6%)	0.616	2 (2%)	12 (12%)	0.007*
21-30 Y	38 (19%)	4 (6%)	34 (25%)	0.001*	19 (20%)	19 (18%)	0.838
31-40 Y	24 (12%)	3 (4.5%)	21 (15%)	0.027*	7 (7%)	17 (8.4%)	0.043*
41-50 Y	33 (16%)	7(11%)	26 (19%)	0.142	15 (15%)	18 (9%)	0.700
51-60 Y	31 (15%)	17(26%)	13(9.5%)	0.001*	19 (20%)	12 (6%)	0.121
>60 Y	28 (14%)	11(17%)	17(12.5%)	0.385	16 (16%)	12 (6%)	0.324

Of E Coli isolated in females 137 (68%) compared to males 65(32%) p-value is 0.00001 (<0.05%). In females' maximum number 34 (25%) were isolated in the reproductive age group, 21-30 yrs, in males' maximum number 17(26%) were isolated in the age group 51-60 yrs which is consistent with other studies. There is no significant difference in the number of isolates obtained from the hospital setting 98 (41%) and community setting 104(51%) the P-value is 0.550 (>0.05). The maximum number of Escherichia coli isolates were obtained in the Adult age group that is 21-30 yrs followed by 41-50 years, least from pediatrics that is from 2-10 yrs. (Table-3).

The study reveals maximum isolates were obtained from an outpatient unit that is 102(50.5%), followed by inpatients. Medical wards showed maximum isolates (50), followed by ICUs (21) and pediatric and surgical wards. The distribution of isolate among various departments was given in the following (table -4).

Table 4: Distribution of E Coli in urine sample as per department wise.

Departments	Total E coli isolates n=202	Percentages
Medical wards	50	24.9%
Surgical wards	3	1.4%
Children ward	11	5.4%
Antenatal ward	15	7.4%
NICU	5	2.5%
PICU	4	2%
MICU	11	5.4%
SICU	1	0.5%
OUT PATIENT	102	50.5%

The Antibiotic testing was carried out using 17 antibiotics which includes beta-lactam, beta-lactam and beta-lactamase inhibitors, Fluoroquinolones, Aminoglycosides Nitrofurantoin and colistin. Among all the antibiotics used, maximum resistance is exhibited to the penicillin group that is ampicillin (81.4%) followed by Norfloxacin (75%), cephalosporins that is cefuroxime (73.26%) and ceftriaxone (67.3%). The least resistance is seen with colistin (1.9%) followed by nitrofurantoin (11.3%) and Meropenem (13.8%).

Among beta-lactamase inhibitors, maximum resistance is seen in Amoxycillin/ clavulanate (65%) followed by Ampicillin/ sulbactam (60%) and piperacillin/ tazobactam (29%). However, in the case of Aminoglycosides, Gentamycin (48%) showed maximum resistance, followed by Amikacin (18%) and Netilmicin (13%). Among fluoroquinolones, maximum resistance is seen in Norfloxacin followed by ciprofloxacin. In carbapenems, maximum resistance is seen in Imipenem (17%) followed by Meropenem (13%). Other antibiotics like Co-trimoxazole showed 50% resistance. (figure1).

Among all the age groups, maximum resistance is seen with ampicillin followed by cephalosporins; minimum resistance is seen with colistin, nitrofurantoin and carbapenems.

The significant difference among sensitivity is seen with cefepime, Norfloxacin, Aminoglycosides and colistin among different age groups. The paediatric age group showed the least resistance to the above drugs. Among males and females' fluoroquinolones showed a significant difference in resistance pattern. It is more resistant in females than males ($p<0.05$). In community and hospital settings, cefepime, colistin and Ampicillin/ sulbactam showed a significant difference in resistance pattern ($p <0.05$). It is more resistant in hospital isolates compared to community-acquired. Overall hospital-acquired isolates showed slightly higher resistance to all the antibiotics compared to community-acquired isolates (Table-5)

Among all the isolates, 28% are MDR, 8% are XDR, and 1.9% are PDR isolates. Among all isolates, 41% are ESBL producers, and 13% are carbapenem-resistant e coli. At the same time, comparison Among different age groups elderly has a higher percentage of resistant isolates than the other two groups. In contrast, among males and females, both have an almost equal percentage of resistant isolates. Among hospital-acquired and community-acquired resistant isolates are more in hospital compared to community-acquired. In department wise percentage of resistant isolates are more in surgical wards followed by ICUs and medical wards (Table -6), (**Figure** -2)

Among resistant isolates, maximum were from females with significant difference ($p =0.001$). Among different age groups, maximum resistant isolates were seen in adults with significant differences ($p= 0. 0001$). While coming to the distribution of resistant isolates in hospital and community settings, it showed a slightly higher percentage from the hospital setting. (Table-7)

Table 5: Distribution of Antibiotics resistant E Coli isolates in different age groups, Gender and Hospital or community-acquired (%)

Antibiotics	n=202 Resistant isolate No (%)	Elderly No 28 (13.9 %)	Adult No 133 (65.8 %)	Pediatric No 41 (20.3 %)	* p	Male, n=65 (32 %)	Female, n= 137 (68%)	* p	Hospital acquired, n=100 (49%)	Community acquired, n= 102 (51%)	* p
Ampicillin	164 (81%)	27 (96%)	104(78%)	33(80 %)	0.08	54(83%)	110(80%)	0.63	85(85%)	79(77%)	0.169
Cefuroxime	148(73%)	24(86 %)	93(70 %)	31(75 %	0.21	48(74%)	100(73%)	0.89	76(76%)	72(71%)	0.384
Ceftriaxone	136 (67%)	23(82 %)	88(66 %)	25(61 %)	0.16	45(59%)	91(66 %)	0.69	73(73%)	63(62%)	0.887
Cefepime	103(51%)	24(86 %)	69(52 %)	10(24 %)	0.001*	31(48%)	72(53 %)	0.51	69(69%)	34(33%)	0.001*
Ampicillin/ Sulbactam	122(60%)	16(57 %)	78(59 %)	28(68 %)	0.50	36(55%)	86(62 %)	0.31	79(79%)	43(42%)	0.001*
Amoxicillin/ clavulanate	132(65%)	22(79 %)	90(68 %)	20(49 %)	0.024	40(62%)	92(67 %)	0.43	61(61%)	72(71%)	0.150
Piperacillin/ tazobactam	59(29%)	8(29 %)	41(31 %)	10(24 %)	0.72	20(31%)	39(28 %)	0.73	30(30%)	29(28%)	0.806
Norfloxacin	145(72%)	21(75 %)	113(85%)	11(27 %)	0.001*	32(49%)	113(82%)	0.0001*	51(51%)	94(92%)	0.001*
Ofloxacin	(55%)	18(64 %)	71(53 %)	23(56 %)	0.49(75%)	63(46 %)	0.	59(59%)	53(52%)	0.	

Gentamycin	97 (48%)	12(43%)	69(52%)	16(39%)	0.29	38(58%)	59(43%)	0.058	42(42%)	55(54%)	0.089
Amikacin	38 (19%)	10(36%)	22(17%)	6(15%)	0.046*	17(26%)	21(15%)	0.065	18(18%)	20(20%)	0.770
Netilimicin	28 (14%)	8(29 %)	20(15%)	0(0%)	<0.05*	12(19%)	16(12%)	0.192	17(17%)	11(11%)	0.201
Imipenem	35 (17%)	5(18 %)	24(18%)	6(15 %)	0.87	16(24%)	19(14%)	0.059	22(22%)	13(13%)	0.822
Meropenem	27 (13%)	6(21 %)	17(13%)	4(10 %)	0.35	11(17%)	16(12%)	0.306	16(16%)	11(11%)	0.276
Nitrofurantoin	23 (11%)	3(11 %)	16(12%)	4(10 %)	0.91	10(15%)	13(10%)	0.217	09(9 %)	14(14%)	0.290
Co-Trimoxazole	101 (50%)	14(50%)	63(47%)	24(59%)	0.45	36(55%)	65(47%)	0.291	54(54%)	47(46%)	0.260
Colistin	4 (2%)	1(2%)	3(2%)	0(0%)	<0.05*	1(2%)	3(2%)	0.756	4(4%)	0(0%)	<0.05*

Table 6: Percentages of resistant isolates in various base lies and demographic characteristics.

	MDR n = 56 (27.8%)	XDR n = 17(8.4%)	PDR n = 4 (1.9%)
Age Elderly n = 28	14(50%)	03(11%)	1(4%)
Adult n = 133	33 (25%)	11(8%)	3(2%)
Pediatrics n = 41	9(22%)	03(7%)	0(0%)
Gender Female n = 137	37(27%)	10(7%)	3(2%)
Male n = 65	19(29%)	07(11%)	1(2%)
Source Hospital acquired n = 100	29 (29%)	10(10%)	4(4%)
Community acquired n = 102	27(26%)	07(7%)	0(0%)
Department wise Medical wards n = 50	15(30%)	04(8%)	0(0%)
Surgical Wards n = 3	3(100%)	2(67%)	2(67%)
ICUs n = 21	13(61%)	04(19%)	2(10%)
OP n = 102	25(24%)	07(7%)	0(0%)

Table 7: Distribution of resistant isolates MDR, XDR and PDR (total 77 isolates) in Uropathogenic E Coli.

Characteristics	Resistant isolates 77	P-value*
Gender Male Female	27(35%) 50(65%)	0.001*
Age Elderly Adult Pediatrics	18(23%) 47(61%) 12(16%)	0.0001*
Source Hospital-acquired Community-acquired	43(56%) 34(44%)	0.091
Department Medical wards Surgical wards ICUs OP	19(25%) 7(9%) 19(25%) 32(41%)	0.005*

n					5 7			0 0 0 1 *			3 1 4

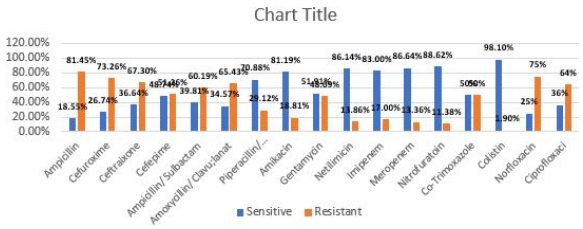

Figure 1: Antimicrobial susceptibility result for 202 E. coli urinary tract isolates in the study period from January 2020 to December 2020.

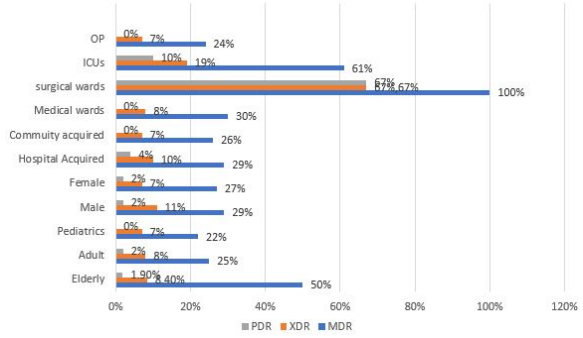

Figure 2. Percentages of MDR, XDR and PDR among Uropathogenic E coli in various base line and demographic characteristics.

Discussion

This study reports bacterial aetiology of UTI-causing organisms. It mainly focuses on the prevalence of uropathogenic *E. coli* and its antimicrobial susceptibility pattern in various baseline and demographic characteristics. Out of 2144 samples received for culture, 531(28%) samples were culture positive. This is similar to other studies conducted by Santhosh. et.al [13]. and Yann Mounaga dzime.et.al [14]. In the present study, among all the isolates, *E. coli* was the most prevalent isolate, 38%, followed by *Enterococcus* 26%. This is consistent with most of the studies like Shivani Gupta. et.al [15]. and Santhosh.et.al [13]. While coming to gender distribution in our study, *E. coli* is more prevalent in women, 68% compared to men. This is similar to most of the studies by Malik. et.al [16]. and Flor Y. Ramírez-Castillo. et al [17]. In females aged 21-30 years, *E. coli* is isolated more, followed by 31-40 years. This is because of their active reproductive age and greater involvement in sexual activity. At the same time, in males, *E. coli* is isolated more in 51-60 yrs this is because, in men of old age, enlargement of the prostate gland,

Use of catheters and neurogenic bladder has been observed, which is similar to another conducted by Malik et al. [16]. In this study, there is no significant difference in percentages of the hospital and community-acquired *E. coli* isolates. This is different from other studies conducted by Flor Y. Ramírez-Castillo et al. [17], where community-acquired *E. coli* percentage is more.

While coming to antibiotic profile analysis carried using 17 antibiotics in our study, maximum resistance is seen with Ampicillin 81% followed by Norfloxacin 75% and cephalosporins 73% minimum resistance is seen with colistin 2% nitrofurantoin 11% and carbapenems 13% this is similar to all the other studies conducted by Malik. et.al [16]. and Niranjan et al. [18]. In our study, there is no significant difference in antibiotic profile with baseline characteristics except with a few antibiotics such as cefepime, Ampicillin/ sulbactam, and colistin which showed a higher resistance percentage in hospital-acquired isolates compared to the community; this is observed in other studies conducted by Flor Y. Ramírez-Castillo. et al [17]. In our study, among 202 *E. coli* isolates, 28% are MDR strains, 8% are XDR, and 1.9% are PDR. This is similar to the study conducted by Silpi et al. [19]. The percentage of MDR in our study is less than in other studies where the MDR percentage is more. In our study, among different wards percentage of MDR isolates is maximum in surgical wards followed by ICUs. This is similar to the study conducted by Silpi et al. [19].

In the present study distribution of resistant isolates is maximum in females compared to males. This is statistically significant (p=0.001). Among different age groups, there is a statistically significant (p= 0.0001) resistant isolate distribution. It is maximum in adults, followed by elderly and pediatrics. The distribution of resistant isolate in hospital and community settings is almost equal. There is no statistically significant difference. This is similar to the study conducted by Flor Y Ramírez-Castillo. et al [16]. While entering IP wards, maximum resistant isolates were from medical and ICUs followed by surgical wards.

Conclusion

The present study highlights *E. coli,* the most leading agent causing UTI, is becoming More resistant with the

Emergence of few pan drug-resistant *E. coli* isolates and an increased percentage of multidrug-resistant and extensively drug-resistant isolates were observed, which is a global concern. So early detection and close monitoring are required to formulate antibiotic policy and avoid antimicrobial misuse. Finally, global efforts are also warranted to develop new antimicrobial agents that can overcome the antimicrobial-resistant organism and minimize the negative clinical outcome and decrease the economic consequences of antibiotic-resistant microorganisms.

What does this study add to existing knowledge?

This study highlights the emergence of MDR, XDR, PDR where stringent infection control measures are required.

Author contribution: Wajid - Supervision, correction of the manuscript, **Saranya** - data analysis, **Pratyusha** - collection of data. **Shazia** - Preparation of manuscript.

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