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Isolation and antibiotic susceptibility of *Escherichia coli* from urine sample received in SMS Medical College, Jaipur

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Abstract
Background: *Escherichia coli* is the most clinically relevant and multiple drug resistant bacterial pathogen causing urinary tract infections (UTIs). In the community, women are more prone to develop UTI. About 20% of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year. In males, with advancing age, the incidence of UTI increases due to prostate enlargement and neurogenic bladder.
Objective: To assess the changing susceptibility pattern of *Escherichia coli* to antimicrobial agents in urine samples.
Material and Method: A total of 160 urine samples collected from patients attending hospital both OPD and IPD. A calibrated loop method (semi quantitative method) was used for the isolation of bacterial pathogens from urinary samples. Significant isolates were identified by conventional methods according to the standard laboratory protocol, including colony morphology, gram staining and biochemical reactions. The antibiotic sensitivity test was performed by modified Kirby Bauer disc diffusion technique.
Result: Among 625 clinically suspected cases of urinary tract infection 250 (40%) were significant growth and 375 (60%) were sterile. Out of 250 positive culture *Escherichia coli* was predominant 160 (64%) among that 92(57%) were ESBL producer while 68(43%) were Non ESBL producer. *Escherichia coli* showed highest sensitivity to Polymixin (100%) followed by Tigicycline (95%).
Conclusion: A high isolation rate of *Escherichia coli* from urine samples of clinically-suspected UTI shows a good correlation between clinical findings. Our study highlights the need for the development of protocol for rational use of antibiotics.

Keywords: antibiotic susceptibility test, *Escherichia coli*, extended spectrum beta lactamase, urinary tract infection

1. Introduction
Escherichia coli is the most clinically relevant and multiple drug resistant bacterial pathogen causing urinary tract infections (UTIs) [1, 2]. It is one of the most common infectious diseases ranking next to upper respiratory tract infection. It has been estimated that 150 million people are infected with UTI per annum worldwide [3]. Urinary tract infection can be a consequence of poor diagnosis and is regarded as the common hospital acquired infection [4, 5]. It is the most common nosocomial infection, accounting for up to 40% of all nosocomial infections [6] while the prevalence of Community-associated UTI is approximately 0.7% in all over the world [7].
In the community, women are more prone to develop UTI. About 20% of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year [8]. It is a universal fact that the prevalence of UTI is more common in females than in males due to their reproductive physiology and pregnancy enhances the occurrence of infection, Female urethra structurally found less effective for preventing the bacterial entry [9].
In males, with advancing age, the incidence of UTI increases due to prostate enlargement and neurogenic bladder[10]. Recurrent infections are common and can lead to irreversible damage of the kidneys, resulting in renal hypertension and renal failure in severe cases [11].
Escherichia coli, the most common member of the family Enterobacteriaceae, accounts for 75 - 90% of all UTIs in both inpatients and outpatients [12] causing both community as well as hospital acquired UTI [13]. *Escherichia coli* present in the gastrointestinal tract as a commensals provide the pool for initiation of UTI and certain serotypes of *Escherichia. coli* responsible for uropathogenicity were traditionally designated as uropathogenic *Escherichia.coli* (UPEC) [14].
Now a day's antibiotics are invariably used for the treatment of UTIs, though resistance to antibiotics has been reported all over the world, particularly in developing countries [15]. Treatment of UTIs is a challenge due to the increasing level of antimicrobial resistance [16]. The prevalence of antimicrobial resistance in patients with UTI is increasing and can vary according to geographical and regional location [17]. Therefore this study is undertaken to isolate and identification of *Escherichia coli* from urine samples received in microbiology laboratory and to determine their susceptibility to commonly available antibiotics which may help the physician to choose appropriate treatment for the prevention of UTI.

2. Material and Method
The present study was conducted in Bacteriology Laboratory of the Department of Microbiology SMS Medical College & Attached Hospital, Jaipur. A total of 160 urine samples collected from patients attending hospital both OPD and IPD were included in the study. A calibrated loop method (semi quantitative method) [18] was used for the isolation of bacterial pathogens from urinary samples. Clean

catch, mid-stream urine samples were received in sterile universal containers. Urine samples were processed within 2 hour of collection and in case of delay, the sample were refrigerated at 2-8°C. The inoculated plates were incubated at 37°C for 24 hour. The number of isolated bacterial colonies was multiplied by 1000 for the estimation of bacterial load/mL of the urine sample. A specimen was considered positive for UTI if growth detected at a concentration of $\geq 10^5$ CFU/mL [19]. Significant isolates were identified by conventional methods according to the standard laboratory protocol, including colony morphology, gram staining and biochemical reactions. The antibiotic sensitivity test was performed by modified Kirby Bauer disc diffusion technique with commercially available Hi-Media antibiotic discs according to Central Laboratory Standard Institute (CLSI) guidelines on Mueller Hinton agar plates. The antibiotics which were used in our study were based on the standard protocol of the hospital and departmental policies. Statistical analysis was performed with the SPSS, Trial version 23. The qualitative data were expressed in proportion and percentages. The difference in proportion was analyzed by using chi square test. Probability P value <0.05 was considered statistically significant.

3. Result

Among 625 clinically suspected cases of urinary tract infection 250 (40%) were significant growth and 375 (60%) were sterile. Out of 625 sample tested 369 (59%) were male while 256 (41%) were female. Out of 250 Positive culture *Escherichia coli* was predominant 160 (64%), followed by *Enterobacter spp* 36(14.4%), *Enterococcus spp*. 22(8.8%), *Klebsiella spp* 14(5.6%) and the least was *Staphylococcus aureus* 03(1.2%). Among the total significant growth, male outnumbered female. Out of 250 positive culture 141(56.4%) were male and 109(43.6%) were female.

Out of 160 isolates of *E. coli* 92(57%) were ESBL producer while 68(43%) were Non ESBL producer among that 58 cases belong to Female and 102 cases belong to Male category and the majority of cases found in the age group of 50 to 59 years which is 26 out of 160 cases. Among males, 18.63% cases were observed in the age group 50 59 years and in females 20.69% cases were observed in 20 to 29 years. *Escherichia coli* showed highest sensitivity to Polymixin (100%) followed by Tigicycline (95%), Fosfomycin (92.5%), Nitrofurantoin (79.3%) while the least sensitive was Ampicillin (4.3%).

Table 1: Total number of sample tested

Urine culture	Number	Percentage (%)
Significant Growth	250	40
Sterile	375	60
Total	625	100.00

Table 2: Distribution of Bacteria in Positive Culture (n=250)

Gram negative Bacteria	Number	Percentage (%)
Escherichia coli	160	64.00
Enterobacter spp	36	14.40
Klebsiella spp	14	5.60
Acinetobacter spp.	06	2.40
Proteus spp.	04	1.60
Gram Positive Bacteria		
Enterococcus spp.	22	8.80
Coagulase negative Staphylococcus	05	2.00
Staphylococcus aureus	03	1.20
Total	250	100.00

Table 3: Distribution of Antibiotic Susceptibility Pattern of *Escherichia coli*

Antibiotic	Resistant	Resistant%	Sensitive	Sensitive%
Ampicilin	153	95.62%	7	4.37%
Norfloxacin	137	85.62%	23	14.37%
Cefotaxime	135	84.37%	25	15.62%
Chloramphenicol	134	83.75%	26	16.25%
Linezolid	132	82.50%	28	17.50%
Cotrimoxazole	122	76.25%	38	23.75%
Ceftazidime	108	67.5%	52	32.5%
Cefoperazone	99	61.87%	61	38.12%
Cefoxitin	91	56.87%	69	43.12%
Piperacillin	81	50.62%	79	49.37%
Nitrofurantoin	33	20.62%	127	79.37%
Fosfomycin	12	7.50%	148	92.50%
Tigecycline	8	5.00%	152	95.0%
Polymyxin	0	0%	160	100%

4. Discussion

Urinary tract infection caused by microbial invasion and subsequent multiplication in urinary tract [20]. In this study we found that most of the cases 369 (59%) were belongs to male than female 256 (41%) because the sample is collected from SMS hospital and not from Mahila Chikitsalaya which is nearby from our teaching hospital.

In this study 625 urine samples were processed, out of which 250 (40%) gave significant growth of pathogens. Our findings are consistent with Sunayana Saha *et al.* [21] (40%) and Singhal A *et al.* [22] (41%). However Prakash D *et al.* [23] (53.82%), Rangari A *et al.* [24] (58.82%) found high prevalence of UTI. There are studies which shows low prevalence of UTI which accounts for 17.7% [25], 18.5% [26],

24.2% [27], 33.3% [28] in India. We found that out of 250 significant growth 134(53.6%) came from out patients (OPD) while 116(46.4%) came from hospitalized patients (IPD). Similar findings were also reported by Surinder K *et al.* [25] (64.61% OPD & 35.38% from IPD). This is supported by the fact that hospitalization is not required for uncomplicated UTI and mostly patients are treated in OPD.

In this study we found that out of 250 significant growth *Escherichia coli* was the most common isolated urinary pathogen (64%). Our result is consistent with various studies [24, 27, 29] but differs from the reports in which *P. aeruginosa* [30] and *Klebsiella* spp [31] were recorded as the predominant bacteria in UTI. Other bacteria isolated from UTI cases in this study were *Enterobacter* spp (14.40%), *Enterococcus spp* (8.80%), *Klebsiella* (5.60%), *Acinetobacter spp.* (2.40%), *Proteus spp*. (1.6%), CONS (2%), *Staphylococcus aureus* (1.2%) Our findings are in accordance with Surinder Kumar *et al.* [25].

In this study we also found that out of 250 cultures positive cases male (56.4%) outnumbered female (43.6%). Similarly out of 160 *Escherichia coli* isolates male were predominant (51.8%) while female were (48.2%) but when we compare with total number of sample tested and isolation rate among male and female we will find the culture positivity rate is more in female (77%) than male (54%) which is significant.

In this study we found that females of the age group 20–39 years were more susceptible (25%) to UTI followed by 50–59 years (16%). Our findings are consistent with various authors Surinder K *et al.* [88] and Mohd Akram *et al.* [28]. These findings correlate with other reports which showed that females are more prone to UTIs than males during adolescence and adulthood [30, 31]. The increasing incidence of UTI in young female is associated with high sexual activity, recent use of a diaphragm with spermicide, and a history of recurrent UTIs [32]. We also found that elderly males (≥50 years) had a higher incidence of UTI (50.76%) when compared with the elderly females (10.59%).

This factor is also reported by other authors whose studies showed that the prostate disease in males is responsible for the increase in incidence of UTI and decrease in female: male ratio in patients above 50 years [33]. Our findings are in agreement with Surinder Kumar *et al.* [25]. and Sujatha RN *et al.* [33].

In this study out of 160 isolates of *E. coli* 92(57%) were ESBL producer while 68(43%) were Non ESBL producer. Our results were in accordance with Wani K A *et al.* [34] where ESBL production was confirmed in 53.4% and Fazlay Bazzaz BS *et al.* [35] where the prevalence of ESBL-producing strains *Escherichia coli* was 59.2%. The high incidence of ESBL production was also reported by Mohanty S *et al.* [36] (60.7%), Rajini E *et al.* [37] (72%) and Sasirekha B *et al.* [38] (61.1%).

Antibiotic susceptibility pattern of microorganisms changing rapidly over a short period. The pattern of antimicrobial resistance of the microorganism causing UTIs vary in their susceptibility to antimicrobials from place to place and from time to time. In this study we observed that the most effective antibiotic for *E.coli* was Polymixin (100%), followed by Tigicycline (95%), Fosfomycin (92.5%) and Nitrofurantoin (79.37%). Most *E. coli* isolates in our study were resistant to ampicillin (95.6%) which resembles other studies Rashed Marandi *et al.* Behroozi *et al.* and Farshad *et al.* followed by Norfloxacin (85.62%), Cefotaxime (84.37%), Chloramphenicol (83.75%), Linezolid (82.50%), and Cotrimoxazole (76.25%)

It has been observed that each study has its own susceptibility pattern of that region, the reason for difference might be factors related to difference in antibiotic use, patient population and prescribing rate [39-41]. The regional variations of resistance to antibiotics may be explained in part by different local antibiotic practices [42].

5. Conclusion

A high isolation rate of *Escherichia coli* from urine samples of clinically-suspected UTI shows a good correlation between clinical findings and microbiological methods.

Our study highlights the need for the development of protocol for rational use of antibiotics and local chemist as well as clinician should be train for importance of rational use of antibiotics.

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