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Research Article

The Prevalent Causative Agent of Urinary Tract Infection and Its Antibiotic Sensitivity Pattern in Islamabad

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Abstract

Background: Urinary Tract Infection (UTI) is common infection typically caused by bacteria accounting for about 25% of all infections in developing countries including Pakistan. It is a major problem and is responsible for not only morbidity but also mortality.

The objectives of this study were to determine the frequency, common causative agents and antimicrobial susceptibility pattern of the most frequently involved pathogen in causation of UTI in patients referred to National Institute of Health (NIH), Islamabad.

Methodology: Clean-catch urine specimens were cultured on blood, CLED and MacConkey agar. Positive specimens were gram-stained and subjected to biochemical reactions for identification of uropathogens. API20E kit was used for confirmation. Biomerieux Vitek 2 system was used for the detection of some rare organisms. AST was performed by Kirby Bauer method also known as Disc Diffusion Technique. Data were analyzed using SPSS version 22.

Results: A total of 395 suspected UTI patients were enrolled in this study. Of which, 112 (28.61%) were positive. Male to female ratio was 1:4.6. Most isolates were Gram-ve bacteria (76.79%). Escherichia coli was found to be the leading causative agent (54.5%), followed by other commonly isolated pathogens. Imipenem was the most effective drug for the treatment of infections caused by nearly all strains of E. coli including ESBL-producing strains (17%). The leading uropathogen had major resistance to amoxicillin clavulanic acid.

Conclusion: The current study revealed that there was marked difference not only in the prevalence but also in resistance pattern shown by E. coli in different genders and groups of age.

Keywords: Urinary Tract Infection; *Enterobacteriaceae*; Biomerieux Vitek 2 System

Abbreviations

UTI: Urinary Tract Infection; AMR: Antimicrobial Resistance; DDST: Extended Spectrum Beta Lactamase; MIC: Multi-resistance; APIE20: Analytical Profile Index for *Enterobacteriaceae*; SPSS: Statistical Package for Social Sciences software

Introduction

Urinary Tract Infection (UTI) is the one of the commonest infections typically caused by bacteria. This infection alone accounts for about 25% of all infections in developing countries [1]. Urinary tract infection is characterized by bacteriuria. The symptoms of this infection are associated with either both parts i.e., upper and lower parts of urinary tract or any of the two [2]. Around the world, Gram negative bacteria belonging to genera of Escherichia and Klebsiella have been reported as the most prevalent UTI causing organisms by several authors [3]. Escherichia coli causes about 70 to 90% of Urinary tract infections [4].

Worldwide estimation was done and it was found that about 6,000,000 patients with UTI visit outpatient departments and around 300,000 are admitted and treated in the hospitals each year [5]. There are about 150 million deaths occurring yearly worldwide [6]. Complicated UTI can result in pyelonephritis which leads to premature births with low weight [7]. Recurrent infections, poor socio-economic status, increasing age, genetic defects, number of intercourses per week, increasing births, diabetes [8]. Certain medical states include sickle cell anemia, deficiency of immune system and Urinary tract abnormalities increase the risk in women [9]. Use of contraceptives also promotes the colonization of coliform bacteria in the periurethral region [10]. UTI is endogenously acquired through catheterization. Catheters allow the entrance of colonic bacteria present on their surface as they act like conduits [11].

Laboratory diagnosis usually begins when empiric therapy fails [12]. Empiric antibiotics have led to rise in multi-drug resistance and fewer options of treatment with high costs [13]. Multi-Drug Resistance (MDR) or multi-resistance resulted because of the changes occurred in the susceptibility pattern of microorganisms towards antimicrobials. Rates of resistance differ in different geographic locations. Antibiotics have different resistance rates due to improper investigation and abuse of drugs. MDR pathogenic bacteria are causing a major health problem [14]. Nosocomial urinary tract infections have increased due to multi-drug resistance in the *Enterobacteriaceae* family for the

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Table 1: Interpretive scheme of biochemical tests for *Enterobacteriaceae* family.

Species	MIU Medium			KIA Medium			
	Motility	Indole	Urea	Slope	Butt	H_2S	Gas
Escherichia coli	+	+	–	Y	Y	–	+
Salmonella typhi	+	–	–	R	Y	+ (weak)	–
Klebsiella pneumonia	–	–	+ (slow)	Y	Y	–	+
Proteus mirabilis	+	–	+	R	Y	+	+
Morganella morganii	+	+	+	R	Y	–	D
Providencia species	+	+	D	R	Y	–	D
Pseudomonas aeruginosa	+	–	D	R	R	–	–

Table 2: Interpretive scheme for sugar, oxidase and citrate tests.

Species	Lactose	Mannose	Glucose	Sucrose	Oxidase	Citrate
Escherichia coli	+	+	+	d	–	–
Salmonella typhi	–	+	+	–	–	–
Klebsiella pneumoniae	+	+	+	+	–	+
Proteus mirabilis	–	–	+	d	–	+
Morganella morganii	–	–	+	–	–	–
Providencia species	–	D	+	d	–	+
Pseudomonas aeruginosa	–	–	D	–	+	+

Keywords: KIA: Kligler Iron Agar; MIU: Motility Indole Urease; H_2 S: Hydrogen sulfide (blackening); R: Red-pink (alkaline reaction); Y: Yellow (acid reaction); d: different strains give different results

last two or three decades worldwide [15].The objectives of this study were to determine the frequency of UTIs, common causative agents and susceptibility pattern of prevalent pathogen in patients visiting Public Health Laboratory Department (PHLD) in National Institute of Health (NIH), Islamabad, Pakistan.

Methodology

A Cross-sectional, quantitative retrospective study was conducted at PHLD, NIH, Islamabad. Therefore, previous data of the year 2018 was collected from Microbiology Department in PHLD. Alongside the patients of last year, patients visiting this year were also enrolled till the end of March 2019.

Inclusion Criteria

Patients of all ages and both genders were selected. All urine samples received in laboratory for culture detection and sensitivity testing were included.

Exclusion Criteria

Patients taking medication at the time of sample collection were excluded and all those urine specimens only for routine examination and microscopy were excluded for this study. Urine specimen with insignificant growth were also not considered.

Sampling and Isolation

The standard procedure adopted by Microbiology Laboratory in P.H.L.D. started with collection of clean catch Midstream Urine Samples (MSU) as explained by Monica Chesbrough Laboratory practice manual, in reception room. 395 samples were received and cultured on Blood agar, MacConkey agar and CLED medium (cystine–lactose-electrolyte-deficient agar or medium). Culture plates were incubated for overnight at 37°C. The bacteria with significant growth were processed for identification and Antibiotic Susceptibility Testing (AST).

Identification of *Escherichia coli*

Cultures with significant growth i.e., $>10^5$ colonies were considered positive specifically for Escherichia coli and cultures with colonies fewer than 10^5 were considered to have insignificant growth and were dealt as negative cultures. Complete identification was done based on Gram reaction, morphology of colony and biochemical characteristics (Koneman EW et al., 2006). On culture, *Escherichia coli* had bright pink colonies as a result of lactose fermentation and they appear as pinkish gram-negative rods under microscope. Biochemical tests were performed i.e., Triple Sugar Iron (TSI) agar, Oxidase, Indole, Citrate utilization and Urease tests. API20E (Biomerieux) is a commercially available kit. It uses 21 biochemical miniaturized tests, available with a database. It was also used to confirm some cases.

Examination of Other Uropathogens

Specimens positive for other uropathogens were also processed the same way as those for *E. coli* were processed. Organisms were Gram-stained and observed microscopically. The differentiation between Genera *Staphylococcus* and *Streptococcus* was done by catalase test and between *Staphylococcus aureus* and other *Staphylococcus* species by coagulase test. DNase test was used for identification of *Staphylococcus aureus*. Differentiation of Gram-negative bacteria was done by biochemical tests used for *Enterobacteriaceae* family and those were:- Indole test to differentiate gram-negative rods, especially *Escherichia coli*, Citrate test, Hydrogen sulfide (H_2S gas) production test to differentiate enterobacteria, *Bacteroides* species and *Brucella* species, Nitrate reduction test to differentiate *Mycobacterium* species and enterobacteria, Oxidase test for identification of *Neisseria, Pasteurella*, and *Pseudomonas* species, Oxidation-fermentation test

Table 3: Distribution of UTI subjects with respect to their age groups.

Age Groups (Years)	No. of Females	%age	No. of Males	%age	Total
0.6	1	1%	0	0	1
1-10	11	12%	0	0	11
11-20	3	3%	1	5	4
21-30	10	11%	2	10	12
31-40	14	15%	2	10	16
41-50	9	10%	2	10	11
51-60	23	25%	4	20	27
61-70	11	12	4	20	15
71-80	10	11	2	10	12
81-90	0	0	3	15	3
Grand total	92	100	20	100	112

for the identification of *Pseudomonas aeruginosa*, Urease test to help identify *Proteus, Morganella* and *Yersinia enterocolitica*. For confirmation, API20E (Biomerieux) kit was used. Multiple tests can be performed on this single kit and the results of each test are converted to digits and these digits are combined to give a numerical code. This code formed is known as "Profile" of the particular organism identified [16]. Biochemical results of Enterobacteria were noted according to scheme given in Medical Laboratory Manual for Tropical Countries shown in Tables 1 and 2.

Biomerieux Vitek 2 system

For reconfirmation of some special and rare organisms, samples were also placed in biomerieux Vitek 2 system. The system provides with complete automation for identifying organism and testing antibiotic sensitivity and can accommodate sixty identification cards at a time [17]. It uses advance colorimetric technology. The principle is based on fluorogenic method and turbidimetric method for identification of organisms and antimicrobial susceptibility testing respectively. Isolates can be identified to the species level [18]. The kits include 64 well Vitek cards: ID-GP (for identifying Gram-positive cocci) ID-GN (for identifying Gram-negative bacilli), AST-GP (for testing sensitivity of Gram-positive bacteria) AST-GN (for testing sensitivity of Gram-negative bacteria) with other reagents. The procedure adopted using Vitek 2 system was according to protocol given by Biomerieux manufacturer. Homogenous suspension of pure colonies in 3.0 ml sterile saline was prepared and the turbidity was adjusted using Densi Check Plus between the range of 0.50- 0.63 McFarland. The suspensions of specimens in test tubes and ID cards with transfer tubes were placed in cassette and the cassette was put inside the vacuum chamber of analyzer machine. Inoculation of ID cards occurred inside the chamber. Transfer tubes were cut through a mechanism used by the Vitek machine. Manually, cassette was transferred to reader-incubator chamber operating at 35.5°C. The interpretation of results was done by databases of ID cards and AST cards available in Vitek computer program.

Antibiotic Susceptibility Testing (AST)

Kirby Bauer method also called Disc Diffusion Technique was used for sensitivity testing as explained by Clinical and Laboratory Standards Institute (CLSI) [14]. This technique is not only simple but also standardized. Suspensions containing about 1-2 × 10^8 colony forming units per milliliter of *E. coli* were prepared and Mueller Hinton Agar plates, having diameter of 150mm, were inoculated [19]. A bacterial lawn was prepared. Next, the commercially available filter-paper disks with fixed concentration of antibiotics were placed on the inoculated Mueller-Hinton agar plates. The plates were then left in incubator at 37°C. After incubation for overnight, the clearance zone around each of the antibiotics was measured according to the Interpretive Standards for *Enterobacteriaceae*. ESBL-producers were phenotypically detected by the key-hole formation through the method DDST [20].

Statistical Analysis

Chi square (χ^2), test for difference between male and female proportions was performed in SPSS version 22. Percentages of uropathogens were also calculated. Chi square was also used for testing difference between proportions of causative agents and for sensitive, resistant and intermediate strains found towards each antibiotic [5]. Susceptibility pattern of *Escherichia coli* was represented graphically.

Results

Prevalence

A total of 395 patients suspected for UTI were enrolled from January, 2018 to March, 2019. Out of which, 112 (28.35%) cases had significant growth i.e.: 112 were positive cases and those cases were subjected to further study. Among positive cases, male to female ratio was 1:4.6. The ages of the subjects ranged from 7 months to 90 years. The uropathogens identified were either Gram-negative or Gram-positive bacteria. Fungal genus involved in causing infection was *Candida*. Mixed cultures were excluded and resampling was done.

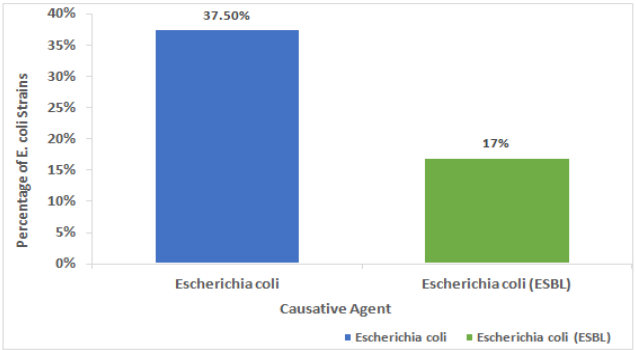

Figure 1: Proportions of non ESBL-producing strains and ESBL-producing strains of *E. coli*.

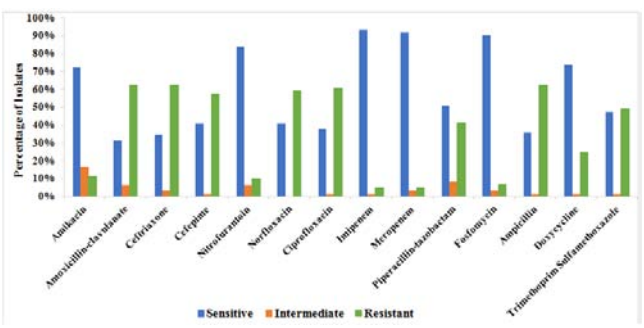

Figure 2: Susceptibility and resistance pattern of *Escherichia coli*.

Table 4: Causative agents and their relative frequencies in UTI patients.

S/N	Major Class	Pathogens	No. of Isolates	%age	P-Value
1	Gram -ve	*Acinetobacter* species	1	0.9	
2	Gram +ve	*Candida* species	5	4.5	
3	Gram +ve	*Enterococcus faecalis*	9	8	
4	Gram -ve	*Escherichia coli*	42	37.5	
5	Gram -ve	*Escherichia coli* (ESBL)	19	17	
6	Gram -ve	*Klebsiella oxytoca*	1	0.9	
7	Gram -ve	*Klebsiella pneumonia*	12	10.7	
8	Gram -ve	*Morganella morganii*	2	1.8	
9	Gram -ve	*Proteus mirabilis*	3	2.7	<0.001
10	Gram -ve	*Providencia Rettgeri*	1	0.9	
11	Gram -ve	*Pseudomonas aeruginosa*	4	3.6	
12	Gram -ve	*Salmonella typhi*	1	0.9	
13	Gram +ve	*Staphylococcus aureus*	2	1.8	
14	Gram +ve	*Staphylococcus aureus* (MRSA)	1	0.9	
15	Gram +ve	*Staphylococcus saprophyticus*	1	0.9	
16	Gram +ve	*Streptococcus agalactiae*	7	6.2	
17	Gram +ve	*Streptococcus pyogenes*	1	0.9	
Grand Total			112	100	

Table 5: No. with respective percentages of sensitive, intermediate and resistant strains of *E. coli* towards Antibiotics.

Antibiotics	Sensitive	Intermediate	Resistant	P-value
Amikacin	44 (72.10%)	10(16.40%)	7 (11.50%)	<0.001
Amoxicillin-clavulanate	19 (31.10%)	4 (6.60%)	38 (62.30%)	<0.001
Ceftriaxone	21 (34.40%)	2 (3.30%)	38 (62.30%)	<0.001
Cefepime	25 (41%)	1 (1.60%)	35 (57.40%)	<0.001
Nitrofurantoin	51 (83.60%)	4 (6.60%)	6 (9.80%)	<0.001
Norfloxacin	25 (41%)	0 (0%)	36 (59%)	0.159
Ciprofloxacin	23 (37.70%)	1 (1.60%)	37 (60.70%)	<0.001
Imipenem	57 (93.40%)	1 (1.60%)	3 (4.90%)	<0.001
Meropenem	56 (91.80%)	2 (3.30%)	3 (4.90%)	<0.001
Piperacillin-tazobactam	31 (50.80%)	5 (8.20%)	25 (41%)	<0.001
Fosfomycin	55 (90.20%)	2 (3.30%)	4 (6.60%)	<0.001
Ampicillin	22 (36.10%)	1 (1.60%)	38 (62.30%)	<0.001
Doxycycline	45 (73.80%)	1 (1.60%)	15 (24.60%)	<0.001
Trimethoprim Sulfamethoxazole	29 (47.50%)	1 (1.60%)	30 (49.20%)	<0.001

54.5% of patients had infections of *Escherichia coli* and 45.5% had infections of other less common uropathogens. A quite considerable frequency was found of ESBL-producing *Escherichia coli*.

Observed Growth Morphology of the Isolates

On Blood Agar Plates (BAPs), most distinctive characteristics of colony of *Escherichia coli* were observed to be whitish grey convex shaped with beta-hemolytic properties of some strains, *Klebsiella pneumonia* showed mucoid and greyish white colonies, Gram-positive bacteria, *Streptococcus agalactiae* had grey or white colonies with clear zone. *Staphylococcus aureus* produced yellow to cream and white colonies occasionally with β-hemolysis in case of some strains, *Candida* appeared in white, creamy and raised colonies.

MacConkey agar is an indicator, selective and differential medium which contains neutral red an indicator which turns pink on acid production. On this medium, *Klebsiella* species and *Escherichia Coli* produced bright pink colonies due to fermentation of lactose resulting in acid production whereas *Proteus, Pseudomonas* produced ammonia through consumption of peptone resulting in colorless or white colonies. *Salmonella typhi* also did not utilize the sugar. Gram-positive bacteria could not grow on this medium due to bile salts and crystal violet.

On CLED (cystine–lactose–electrolyte-deficient agar or medium),

containing bromothymol blue-pH indicator which changes its color from green to yellow in the presence of lactose fermenters, *Escherichia coli* had deep yellow-centered colonies. Colonies of *Klebsiella* species were mucoid, yellow to whitish. *Pseudomonas aeruginosa* appeared to have green, matted-surfaced colonies with rough periphery. Gram-positive organisms also showed growth. Colonies of *Staphylococcus aureus* and Group-D *Streptococci* appeared yellow like those of *Escherichia coli*.

Gender and age-group wise distribution of UTI

The ages of the subjects were between 7 months to 90 years. This large interval was divided into 10 smaller intervals (age groups) to find out the relationship between age and incidence of infection. For both genders, incidence of Urinary tract infection was highest in the age-group: 51 to 60 years. Out of 112 patients, 27 (24%) cases belonged to this group. Out of 27, 23 were females and 04 were males. Out of total 92 females, 23 (25%) were found in this group and out of 20 males, 04 (20%) males were in this group followed by the same incidence in the age group of 61 to 70 years. The next age group with high prevalence i.e. 14 (15%), in females was 31-40 years while for males it was 81-90 years i.e. 3 (15%) (Table 3).

Chi square test for difference between male and female proportions was applied and the results were: χ^2 (46.286[a]), degrees of freedom (1), P-value (<0.001). The probability value was statistically significant, which means that there exists difference between proportions of infected males and females because there was a significant difference between the two proportions. Majority of the infected subjects were females 92 (82%) out of the total of 112 patients.

Frequencies of Isolates (Uropathogens)

Among the positive patients, isolated Gram-negative bacteria were 86 (76.79%), Gram-positive were 21 (18.75%) and fungal positive cultures i.e., *Candida* species were 5 (4.46%). The result of Chi-square test for the difference in proportions of causative agents was: chi square (262.00), degrees of freedom (16), P-value (<0.001). Probability value obtained was statistically significant. Most prevalent pathogen identified was *Escherichia coli*. Out of 112 agents, 42 (37.50%) were *E. coli* with a quite considerable separate frequency of ESBL (Extended Spectrum β-Lactamase) producing-*Escherichia coli* 19 (17%), followed by *Klebsiella pneumonia* 12 (10.70%), *Streptococcus faecalis* 9 (8%), *Streptococcus agalactiae* 7 (6.20%) (Table 4).

Results of AST for *E. coli*

Examination of Mueller Hinton Agar plates with antibiotics was done after an incubation of 24 hours. The major pathogen detected was *Escherichia coli*. In more than half of the subjects, *E. coli* was the causative agent i.e., 54.5% of patients under study had infections of *Escherichia coli* with significant proportion of ESBL-producing *E. coli* resistant to penicillin, β-lactam antibiotics (cefepime and ceftriaxone). Out of 19 cultures positive for *E. coli* (ESBL), 16 were from females with mean age of 43.63 years and only 3 cultures were from males with age mean 57.67 years. Out of total, 61 (42+19) strains of *Escherichia coli*, 68.9% were non ESBL producing and 31.15% were ESBL producing *Escherichia Coli* (Figure 1).

Chi-square test for difference between proportions was applied, except for norfloxacin, the p-value was <0.001 for each antibiotic, which is smaller than 0.05 so the null hypothesis of equal proportions was rejected, which means proportions of sensitive, intermediate and resistant strains differed for each antibiotic. This study revealed that the causative strains of most leading organism *Escherichia coli* showed the highest resistance to amoxicillin-clavulanate, ceftriaxone and ampicillin (62.30%), followed by ciprofloxacin (60.70%), norfloxacin (59%) and cefepime (57.40%), trimethoprim sulfamethoxazole (49.20%), piperacillin-tazobactam (41 %) and lowest resistance to imipenem and meropenem (4.90%). Most strains were intermediate to amikacin (16.40%). Effective drug, towards which *Escherichia coli* was most susceptible, was imipenem (93.40%) followed by meropenem (91.80%), fosfomycin (90.20%), nitrofurantoin (83.60%), doxycycline (73.80%), amikacin (72.10%) (Table 5), (Figure 2).

Resistance Shown By Different Age Groups

The prevalent pathogen *Escherichia coli* was most resistant towards amoxicillin-clavulanate, ceftriaxone, ampicillin, next to ciprofloxacin, norfloxacin and then to cefepime. The age group of patients showed highest resistance was 51-60 years towards all these antibiotics. In this age group (51-60 years), there were 11 (41%) patients out of the total 27 patients of four high resistance showing groups for Amoxicillin-clavulanate, 9 (33.33%) patients out of the total 27 patients of four high resistance showing groups for Ceftriaxone, 9 (31%) patients out of the total 29 patients of five high resistance showing groups for Ciprofloxacin, 9 (36%) subjects out of the total 25 patients of four high resistance showing groups for Norfloxacin and Cefepime. In the same age groups 10 (90.9%) out of 11(41%) patients were resistant females and only 1 (9.09%) was resistant male for Amoxicillin-clavulanate whereas, 8 (89%) out of the total 09 resistant patients were females and only 1 (11%) was male for every other antibiotic (ceftriaxone, ciprofloxacin, norfloxacin and cefepime. Males comparatively had high resistance in elderly age groups (Table 6).

Discussion

In the past, many researchers have been undertaken for determination of prevalence of Urinary Tract Infections (UTI) and the possible uropathogens. The disease does not seem threatening but it still accounts for morbidity and mortality. This infection alone accounts for about 25% of all infections [21]. In a study, 182 (20.73%) cases were positive out of 878 [1] and in our study, 112 (28.35%) cases had significant growth. The prevalence reported in a study in India was 117 (76.29%) positive subjects out of 232 [22]. In another study previously held in National Institute of Health, 83 (74.10%) were positive out of 115 specimens for urinary tract infection with male to female ratio 34:81 [5]. Its results of prevalence were dissimilar to our study whereas results of male to female ratio were similar to this study as we had 20:92 male to female ratio.

Muthulakshmi and Gopalakrishnan (2017), reported 44% females belonged to the age group 15-24 years but our study showed most of the infected females (23%) were in group of 51-60 years [23]. Among males, there was more incidence of UTI according to one study conducted in Bangladesh in older age group 50-90 (23.64%) than in younger groups (8.18%) [24]. This agrees with the results our study produced. We also found high percentage (65%) of males aged between 51-60 years as compared to percentage (35%) of males belonging to younger age groups. The results of this research do not seem to corroborate the previous evidence in which cases of boys (15.2%) were more than those of girls (6.6%) having age less than 15

Table 6: Age groups showing highest resistance.

Antibiotics	Age group	No. of resistant *E. coli*	Male	Female
Amoxicillin-Clavulanate (AMC)	1-10	7 (26%)	0 (0%)	7 (100%)
	41-50	4 (15%)	0 (0%)	4 (100%)
	51-60	11 (41%)	1 (9.09%)	10 (90.9%)
	61-70	5 (18%)	3 (60%)	2 (40%)
		27 (100%)	4 (14.81%)	23 (85.19%)
Ceftriaxone (CRO)	1-10	6 (22.22%)	0 (0%)	6 (100%)
	41-50	6 (22.22%)	0 (0%)	6 (100%)
	51-60	9 (33.33%)	1 (11%)	8 (89.9%)
	61-70	6 (22.22%)	4 (67%)	2 (33%)
		27 (100%)	5 (18.52%)	22 (81.48%)
Ciprofloxacin (CIP)	1-10	4 (14%)	0 (0%)	4 (100%)
	31-40	4 (14%)	2 (50%)	2 (50%)
	41-50	5 (17%)	0 (0%)	5 (100%)
	51-60	9 (31%)	1 (11%)	8 (89%)
	61-70	7 (24%)	4 (57%)	3 (43%)
		29 (100%)	7 (24.14%)	22 (75.86%)
Norfloxacin (NOR)	1-10	5 (20%)	0 (0%)	5 (100%)
	41-50	4 (16%)	0 (0%)	4 (100%)
	51-60	9 (36%)	1 (11%)	8 (89%)
	61-70	7 (28%)	4 (57%)	3 (43%)
		25 (100%)	5 (20%)	20 (80%)
Cefepime (FEP)	1-10	6 (24%)	0 (0%)	6 (100%)
	41-50	6 (24%)	0 (0%)	6 (100%)
	51-60	9 (36%)	1 (11%)	8 (89%)
	61-70	4 (16%)	3 (75%)	1 (25%)
		25 (100%)	4 (16%)	21 (84%)

years [25]. We had 0% boys and 100% girls with ages between 1-10 years. The agents involved in causation of infections are usually Gram -ve organisms. The rate revealed by this study was 76.79%. Similar were the results of work done by Moges and Genetu (2002), reported the rate of 71.5% for Gram -ve bacteria [15].

Similar to our findings, many researchers have found *Escherichia coli* to be the most prevalent pathogen globally. Like our study, many other studies held earlier in Pakistan reported *E. coli* to be the predominant causative agent with the percentages 73.1%, 48%, 66% respectively [26,27,28]. Previously, study was done in Kohat region of Pakistan and the percentage of *E. coli* was 41.4% followed by *Klebsiella pneumonia* (15.5%) [14]. We also found the highest percentage of *Escherichia coli* (54.5%) which was followed by *Klebsiella pneumonia* (10.70%). A study was conducted in Egypt to find out the possible role of ESBL-producing *E. coli* in causation of Urinary tract infections. They found 36 (36%) of ESBL-producers out of 100 strains of *Escherichia coli* and 64 (64%) were non ESBL-producers [29]. Similarly, our findings were 19 (31.15%) ESBL-producers out of 61 strains of *E. coli* and 42 (68.9%) were non ESBL-producers and this is a considerable frequency.

Third prevalent uropathogen after *K. pneumonia* in our study was Gram-positive bacteria *Streptococcus faecalis* a group-D Enterococcus (8%). This substantiates the previous findings in the literature [30]. Some other studies showed different third pathogen. In one study, the third prevalent pathogen was from the genus Proteus (10%) [31]. In another study, the third pathogen was *Staphylococcus saprophyticus* (6.4%) [32]. *Staphylococcus saprophyticus* is considered the second most prevalent causative bacteria for urinary tract infection in females aged 18-39 years [33]. Unexpectedly, only one (0.90%) *Staphylococcus saprophyticus* was identified in this current study.

Some rare pathogens associated with urinary tract infections were also identified and those were Gram-ve bacteria *Providencia rettgeri* (0.90%) and *Morganella Morganii* (1.80%) in this study. On the contrary, rare pathogens identified were Acinetobacter calcoaceticus (1.17%) and *Citrobacter freundi* (1.17%) in another research [34]. Fungal infections also occur. Urinary tract infection is mostly caused by Candida species because of frequent catheterization and receiving broad spectrum antibiotics [35]. We found considerable frequency of infections i.e., 5 (4.50%).

One of the objectives of this research was determination of susceptibility of the most frequent causative Gram-ve bacteria *Escherichia coli*. More than half of the subjects (54.5%) had infections

of *E. coli*. Most of the strains including ESBL producing strains in our subjects were sensitive to imipenem (93.40%) and meropenem (91.80%) and this corresponds to the study that showed *E. coli* to be highly sensitive to meropenem, imipenem and Amikacin (100%) [24]. Although the third drug to which *E. coli* was highly sensitive was fosfomycin (90.20%) instead of amikacin in our study. Farhana Akter et al., (2012) also reported that Imipenem was observed to be an effective drug. They found 94.48% organisms sensitive towards it. 83.60% of isolates were sensitive to nitrofurantoin in the current study [36]. Similarly, 85.19% were sensitive in another study [37]. According to Kibret and Abera, (2011), high rate of resistance was observed to amoxicillin (86.0%) and this agrees with what this study showed, 63.30% was the rate of resistance [38]. We found higher value of sensitivity i.e., more strains were sensitive to ampicillin (36.10%) in contrast to earlier findings in which only 7.61% strains were sensitive [39].

Beta-lactamase inhibitors are the antibiotics which have been developed due to emergence of resistance and are used to treat infections caused by ESBL-producing *E. coli*. A Beta-lactamase inhibitor contains two parts: lactamase-sensitive part and the other part which inhibits the action of Beta-lactamase produced by these bacteria. The examples of these inhibitors include piperacillin-tazobactam, amoxicillin-clavulanic acid etc. Piperacillin and amoxicillin are susceptible to Beta-lactamase producers and are easily attacked by beta-lactamases whereas their combination with the groups tazobactam and clavulanate respectively renders these enzymes inactive by binding irreversibly to them [40].

The above-mentioned study, reported that their isolates showed high sensitivity towards tazocin (87.5%), which is active combination of piperacillin and tazobactam [40]. Our finding is in contradiction with this study. We had 13 (68.42%) resistant ESBL-producers towards piperacillin-tazobactam out of the 19 (100%). The reason behind is that the species of *Escherichia* and *Klebsiella* are probably capable of producing many types of Beta-lactamases [41]. All ESBL isolates were resistant to amoxicillin-clavulanate and third generation cephalosporins and this confirms the evidence given by some researches held before [42,43,27].

Resistance varies across the age groups and there is also variation gender wise. A study was done and it showed that there is lower susceptibility of *E. coli* in males who belong to older age groups towards antibiotics. *E. coli* strains in males were lesser sensitive to amoxicillin-clavulanate between 18 to 64 years and above 64 years i.e., 55.6% in males and 67.5% in females and 57.0% in males and 64.2% in females respectively [44], which means that males were more resistant and that is what our study confirms. In age group of 61 to 70 years, we found males more resistant (60%) as compared to females (40%) not only for amoxicillin-clavulanate but also for other antibiotics.

Conclusion

Urinary tract Infections remain to be a problem. Although uncomplicated infections can be treated but recurrent infections can lead to complications. The bacterial resistance halts the appropriate treatment of infection. Bacteria especially from *Enterobacteriaceae* family are becoming resistant. This study focuses on *Escherichia coli* as it was the most prevalent uropathogen. This work has also gone some way towards better understanding of infection. The empiric therapy can itself cause increase in mutants. Appropriate treatment is necessary. The current research shows that there exists an association between gender and disease. The proportion of females was remarkably high and the frequency was also high in children under 10 years old. The resistance pattern of *E. coli* differed with different age groups. Although frequency of male subjects was low, but there was more resistance observed in elderly ages in males as compared to females.

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