ANTI-MICROBIAL RESISTANCE IN AGENTS CAUSING URINARY TRACT INFECTIONS

Zuhair Ali Rizvi
Rawalpindi Medical University
dr.zarizvi@gmail.com

Ali Murad Jamal
Rawalpindi Medical University
alimuradjamal@hotmail.com

Ali Hassan Malik
Rawalpindi Medical University
alihassanmalik05@hotmail.com

Naimat Ullah
Rawalpindi Medical University
naimatullah9976@gmail.com

Daneyal Arshad
Rawalpindi Medical University
bravesoldier2014@gmail.com

ABSTRACT

BACKGROUND:

Urinary Tract Infections are usually treated with empirical therapy by physicians based on previous knowledge of predictability of causative agents and their antimicrobial susceptibilities.

OBJECTIVE:

The objective of this study was to determine the frequency of various pathogens causing urinary tract infections and their antimicrobial susceptibility in patients presenting in out-patient department of a tertiary care hospital.

MATERIALS AND METHODS:

This descriptive cross sectional study was conducted in Out-Patient department of Urology of Benazir Bhutto Hospital during a period of 6 months from January 2017 to June 2017 after ethical approval from institutional research forum of Rawalpindi Medical University. 1000 patients (12 years old or above) that were clinically suspected for urinary tract infections were included in this study. Patients with co-morbidities like diabetes mellitus, renal pathologies, Immunodeficiency disorders, malignancies and congenital urogenital disorders were also excluded. Recipients of corticosteroid therapy or with a history of intake of broad spectrum antibiotics in previous 15 days were also excluded. Modified Kirby-Bauer disc diffusion method was used for determining the antimicrobial resistance against various antimicrobials.

RESULTS:

A total of 530 (53%) isolates were found to be culture positive for E.coli(77.4%), Klebsiella (6.4%), Enterobacter (6.0%), Pseudomonas (3.8%), Staphylococcus saprophyticus (3.4%), Citrobacter (1.1%) and Morganella (0.4%).

Antimicrobial resistance against commonly used antimicrobials was alarmingly high

CONCLUSION:

Surveillance of trends of antimicrobial susceptibility pattern is highly important.

Keywords: Urinary Tract Infections, UTI, Agents, Anti-microbial susceptibility

INTRODUCTION:

Urinary Tract Infections (UTI) are one of the most commonly diagnosed diseases in Out-Patient Department. (1) The selection of antibiotic therapy by a physician is based on knowledge of prevalent microorganisms in the setting and recent updates about the antimicrobial susceptibility patterns and the clinical status of the patient. (2)

Although E. coli is the most commonly isolated microorganisms from patients with UTI, however a significant difference in the prevalence has been stated. The percentage isolation of E. coli varied from 26.1% in a Turkish study to 55.1% in a developing country like India. (3, 4)

Patients with diabetes are at four times more risk of developing Urinary Tract as compared to non-diabetics (5)

High resistance of agents causing UTI against commonly used antimicrobials has been reported. In a study conducted in Ethiopia, it has been stated that 93.3%, of the isolates were sensitive to gentamicin. Similarly, 60%, 60%, 56.6%, 46.6%, 40%, 33.3%, of the isolates were sensitive to chloramphenicol, nitrofurantoin, ciprofloxacin, Trimethprime-Sulfamethoxazole (TMP-SMX), ceftriaxone and nalidixic acid, respectively. (6)

Therefore, there was a need to conduct a study to determine most common agents associated with Urinary Tract Infections and their current antimicrobial resistance patterns so as to formulate a better antimicrobial therapy.

The objective of this study was to determine frequency of Urinary Tract Infections, agents causing UTI and their current antimicrobial resistance profiles.

MATERIALS & METHODS:

This descriptive cross sectional study was conducted among patients presenting in Out-Patient Department of Urology of Benazir Bhutto Hospital, Rawalpindi, after ethical approval from Institutional Research Forum of Rawalpindi Medical University.

Patients above the age of 12, presenting in above stated out-patient departments and clinically suspected for Urinary Tract Infections (UTI) were included in this study.

Patients who had no symptoms related to UTI were excluded. Patients with co-morbidities like diabetes mellitus, renal pathologies, Immunodeficiency disorders, malignancies and congenital urogenital disorders were also excluded on the basis of history. Recipients of corticosteroid therapy or with a history of intake of broad spectrum antibiotics in previous 15 days were also excluded.

Non cooperative female patients who didn't give consent or refused to provide necessary information were also excluded.

The subjects included in the study were provided with a wide-mouthed standard sized sterile container. They were advised to clean the area around urethra with water before collecting the sample, let the area dry and collect the sample by catching the mid-stream with container being held at 2-3 inches away.

50 µl of uncentrifuged urine was taken on a clean slide and a cover slip was placed on it. The slide was then viewed under microscope. Presence of blood cells, epithelial cells, pus cells or cast bodies was duly noted. The presence of ten or more pus cells per high power field was considered as significant pyuria.

Gram staining technique was employed. Detection of at least one or more morphologically similar bacteria per oil immersion field was called significant.

10µL of specimen was transferred to MacConkey's agar plate using a calibrated loop method. The agar plates were incubated at 35-37°C for 24 hours for identification of lactose fermenting and non-lactose fermenting bacteria. A specimen was considered positive for UTI if a single organism was cultured at a concentration of 10⁵ Colony Forming Units/ml.

CLED (Cystein Lactose Electrolyte Deficient) medium was further used for identification and isolation of urinary pathogens.

Modified Kirby-Bauer disc diffusion method was used for determining the antimicrobial susceptibility. The colonies were placed on agar plates using sterile inoculating wire loop. Antibiotic disks were placed using sterile forcep. The plates were left for 1 hour at room temperature to allow diffusion of antibiotics from the disks. The agar plates were again incubated for 24 hours at 37°C. CLSI guidelines were used to determine antimicrobial susceptibility.

Antimicrobial susceptibility and resistance was tested against Ampicillin, Amoxicillin + Clavulanic Acid (AMC), Gentamycin, Amikacin, Cefoperazone, Ceftazidime, Cefixime, Ceftriaxone, Cefepime, Piperacillin+Tazobactam (TZP), Cefoperazone+Sulbactam (CFP+SUL), Carbapenem, Fosfomycin, Trimethoprim/sulfamethoxazole (SXT), Ciprofloxacin, Ofloxacin, Levofloxacin, Norfloxacin and Nitrofurantoin. Data was analyzed using SPSS v22.0 and descriptive statistics were applied.

Data was entered and analyzed using SPSS v22.0. Following Operational Definitions were used:

- Microscopy findings of more than 10 WBC per high power field were called significant pyuria.
- Significant bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of more than 100,000 cfu/ml.

RESULTS:

Out of 1000 patients, 303 (30.3%) males and 697 (69.7%) females, who were clinically suspected for Urinary Tract Infection, 530 (53%) patients, 160 (52.8%) males and 370 (53.0%) females were found to be culture positive while 430 (43.0%) patients, 143 (47.2%) males and 327 (47%) females, were culture negative.

Of 530 culture positive specimens, *E.coli* was most frequently isolated followed by *Klebsiella spp.*, *Enterobacter spp.*, *Pseudomonas spp.*, *Staphylococcus spp.*, *Proteus spp.*, *Citrobacter spp.*, and *Morganella spp.* The difference in gender wise distribution of various pathogens was statistically significant $p < 0.05$. (Table 1)

Table 1: Frequency of isolation of various microorganisms from urinary samples.

ORGANISM	NUMBER	% AGE	MALE		FEMALE	
			N	%	N	%
<i>E. coli</i>	410	77.4	116	72.5	294	79.5
<i>Klebsiella spp.</i>	34	6.4	10	6.2	24	6.5
<i>Enterobacter spp.</i>	32	6	4	2.5	28	7.6
<i>Pseudomonas spp.</i>	20	3.8	14	8.8	6	1.6
<i>Staphylococcus spp.</i>	18	3.4	10	6.2	8	2.2
<i>Proteus spp.</i>	8	1.5	4	2.5	4	1.1
<i>Citrobacter spp.</i>	6	1.1	2	1.2	4	1.1
<i>Morganella spp.</i>	2	0.4	0	0.0	2	0.5

To test the null hypothesis that the “Distribution of Age is same across all categories of Organism”, Independent Samples Kruskal-Wallis test was applied. $P < 0.05$ was considered

significant .The null hypothesis was rejected ($p=0.047$). The distribution of age among various organisms was not same.

To compare mean age of isolation of various pathogens in males and females, independent sample “t” test was applied. $P<0.05$ was considered significant. The difference in means of isolation of various pathogens as per gender was significant for *E. coli*, *Enterobacter spp.*, *Staphylococcus spp.* and *Citrobacter spp.* (Table 2)

Table 2: Mean age of distribution of various microorganisms in context with gender.

ORGANISM	MEAN AGE	MALE	FEMALE	Independent sample “t” test
		N	N	P value
<i>E. coli</i>	47.34	53.82	44.66	$P=<0.0001$
<i>Klebsiella spp.</i>	42.65	52.00	38.75	0.0611
<i>Enterobacter spp.</i>	46.50	73.50	42.64	0.0056
<i>Pseudomonas spp.</i>	62.80	59.43	70.67	0.0913
<i>Staphylococcus spp.</i>	47.22	56.80	35.25	0.0040
<i>Proteus spp.</i>	40.75	31.50	50.00	0.2324
<i>Citrobacter spp.</i>	54.67	39.00	62.50	0.0004
<i>Morganella spp.</i>	43.00	43.00	-	-
Independent Samples Kruskal-Wallis test	$P = 0.047$			

162 Antimicrobial resistance of various pathogens against Ampicillin, Amoxicillin + Clavulanic Acid
 163 (AMC), Gentamycin, Amikacin, Cefoperazone, Ceftazidime, Cefixime, Ceftriaxone, Cefepime,
 164 Piperacillin+Tazobactam (TZP), Cefoparazone+Sulbactam (CFP+SUL), Carbepenam,
 165 Fosfomycin, Trimethoprim/sulfamethoxazole (SXT), Ciprofloxacin , Ofloxacin, Levofloxacin,
 166 Norfloxacin and Nitrofurantoin was determined. (Table 3)

167

168

169

170

171

172

173

174

175

176

177

178

179 Table 3: Anti-microbial resistance in various microorganisms against commonly used drugs.

Antimicrobial Agent	<i>E. coli</i>	<i>Klebsiella spp.</i>	<i>Enterobacter spp.</i>	<i>Pseudomonas spp.</i>	<i>Staphylococcus spp.</i>	<i>Proteus spp.</i>	<i>Citrobacter spp.</i>	<i>Morganella spp.</i>
Ampicillin	87.3	100.0	50.0	0.0	100.0	100.0	100.0	100.0
AMC	62.7	100.0	50.0	.	0.0	2.0	100.0	100.0
Gentamycin	31.0	43.8	86.7	40.0	12.5	33.3	66.7	0.0
Amikacin	1.0	25.0	0.0	20.0	0.0	33.3	0.0	0.0
Cefoparazone	100.0	100.0	100.0	20.0	.	100.0	0.0	0.0
Ceftazidime	.	.	.	22.2	.	50.0	0.0	.
Cefixime	66.0	50.0	66.7	91.7	.	25.0	0.0	0.0
Ceftriaxone	63.7	47.1	66.7	100.0	.	25.0	0.0	0.0
Cefepime	63.2	43.8	66.7	22.2	.	0.0	0.0	0.0
TZP	11.3	17.6	33.3	22.2	.	0.0	66.7	0.0
CFP+SUL	10.3	29.4	33.3	22.2	.	0.0	66.7	0.0
Carbepenam	2.5	5.9	16.7	22.2	.	33.3	0.0	0.0
Fosfomycin	2.0	0.0	20.0	.	33.3	100.0	33.3	100.0
SXT	71.9	52.9	66.7	.	33.3	25.0	66.7	0.0
Ciporfloxacin	75.4	47.1	87.5	60.0	.	.	33.3	0.0
Ofloxacin	100.0	.	.	70.0	.	.	0.0	.
Levofloxacin	93.3	88.9	100.0	77.8	75.0	50.0	.	.
Norfloxacin	75.4	40.0	0.0	70.0	42.9	33.3	66.7	0.0
Nitrofurantoin	10.7	70.6	0.0	66.7	0.0	100.0	33.3	100.0

DISCUSSION:

In a study conducted in Iran, It has been stated that of all the urinary samples included in the study, 70.3% belonged to female patients while 29.7% belonged to male patients which is in accordance with our study. We observed that 69.7% belonged to female while 30.3% samples belonged to male patients. (1) It has also been stated by the same study that the difference between gender-wise distribution of various pathogens is statistically significant ($p < 0.05$) which is also in accordance with our study where $p < 0.05$. (7)

Urinary Tract Infections are becoming difficult to treat owing to increasing resistance (8)

Emergence of resistance against broad spectrum antibiotic agents such as extended spectrum Beta Lactams, Fluoroquinolones and Carbapenams is causing problems worldwide. (9)

E.coli is the most commonly isolated pathogen from urinary samples. In a study conducted in United States of America, it was observed that 53.6% samples were culture positive for E. coli, 14.6% for Proteus, 13.9% for Klebsiella, 4.5% for Enterococcus and 4.1% for Staphylococcus. (10)

According to the same study, 60 % strains of E. coli were resistant to Fluoroquinolones, 7% to Nitrofurantoin, and 27% to SXT. (4) while according to our study, 75.4% to 100% strains of E. coli were resistant to Fluoroquinolones, 10.7% to Nitrofurantoin and 71.9% to SXT (10)

In another study, it was observed that 60% of isolates from urinary samples were of E.coli, 12% of Klebsiella and 8% for Enterococcus. (11)

200 In a study conducted in India, it was observed that frequency rate for E.coli was 69.8% followed
 201 by Klebsiella to be 7.9%, 4.8% for Staphylococcus, 4.8% for Pseudomonas, 4.8% for
 202 Enterococcus and 1.6% for MRSA. (12) These results were comparable to our observations

203 It was further stated that 81.8% strains of E.coli were resistant to Ampicillin, 72.7% to AMC,
 204 54.5% to Cefepime, 21.5% to (CFP+SUL), 2.3% to 6.8% to Carbapenams, 36.4% to Gentamicin,
 205 9.1% to Amikacin, 65.9% to Ciprofloxacin, 61.4% to Levofloxacin, 47.7% to SXT and 15.9% to
 206 Nitrofurantoin. These resistance rates were also comparable to our findings. (12)

207 According to a study, only 57% individuals out of those with provisional diagnosis of Urinary
 208 Tract Infections were culture positive for Uropathogens which is comparable to 53% in our
 209 study. (13)

210 According to a study conducted in Indonesia, it was observed that resistance of E. coli against
 211 Fluoroquinolones ranged from 41.4% for Levofloxacin to 71.6% for Ciprofloxacin while 78.4%
 212 isolates of Klebsiella were resistant to Levofloxacin. Less than 5% strains of E.coli and Less than
 213 20% strains of Klebsiella were resistant to Fosfomycin.

214 It was observed that 85.5% strains of E.coli were found to be resistant to Ampicillin, 50.5% to
 215 AMC, 32.0% to Gentamicin, 58.1% to Ceftriaxone, 2.5% for Carbapenams, 0.9% to Fosfomycin
 216 and 13.1% to Nitrofurantoin while according to our study values were 87.3%, 62.7%, 31.0%,
 217 63.7%, 2.5%, 2.0% and 10.7 % respectively.

218 It was also stated that 40.5% strains of Klebsiella were resistant to Gentamicin, 5.4% to
 219 Carbapenams, 78.4% to Levofloxacin, 83.8% to Nitrofurantoin while according to our
 220 observations, resistance rates were 43.8%, 5.9%, 88.9% and 70.6% respectively. (14)

Ilić et al has stated that frequency of E.coli was isolated from 67.7% samples and 69.5% of strains of E.coli were found to be resistant to Ampicillin which is in accordance with our study. (15)

An American study has stated that resistance among isolates of E.coli from urinary samples was lowest for Nitrofurantoin (<1%). But according to our observations, resistance rates of E.coli against Nitrofurantoin were found to be 10.7%. (16)

Our study is also in agreement with previously done studies which state that 61% strains of Klebsiella were resistant to Extended Spectrum Beta Lactams. (17)

In a few other studies, the resistance rates of E. coli against Nitrofurantoin have been stated to be less than 15% which is in accordance with our study. (18,19)

REFERENCES:

1. Gales AC, et al. Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program (1998) J Antimicrob Chemother. 2000;45(3):295–303
2. Haller M, Brandis M, Berner R. Antibiotic resistance of urinary tract pathogens and rationale for empirical intravenous therapy. *Pediatr Nephrol*. 2004;19:982–986
3. Kuzdan C, Soysal A, Culha G, Altinkanat G, Soyletir G, Bakir M. Three-year study of health care-associated infections in a Turkish pediatric ward. *J Infect Dev Ctries*. 2014;8:1415–1420
4. Gupta S, Kapur S, Padmavathi D. Comparative prevalence of antimicrobial resistance in community-acquired urinary tract infection cases from representative States of northern and southern India. *J Clin Diagn Res*. 2014;8(9):DC09–12
5. Muller LM, Gorter KJ, Hak E, et al. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. *Clin Infect Dis*. 2005;41(3):281–288

6. Seifu WD, Gebissa AD. Prevalence and antibiotic susceptibility of Uropathogens from cases of urinary tract infections (UTI) in Shashemene referral hospital, Ethiopia. *BMC Infectious Diseases*. 2018;18:30
7. Pouladfar G, Basiratnia M, Anvarinejad M, Abbasi P, Amirmoezi F, Zare S. The antibiotic susceptibility patterns of uropathogens among children with urinary tract infection in Shiraz. Janczewska. E, ed. *Medicine*. 2017;96(37):e7834.
8. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect Dis Clin North Am*. 2014;28:1–13. This paper presents the most recent information about UTIs and their socioeconomic impact
9. Chen YH, Ko WC, Hsueh PR. Emerging resistance problems and future perspectives in pharmacotherapy for complicated urinary tract infections. *Expert Opin Pharmacother*. 2013;14:587–596. This paper highlights the emerging resistance among bacterial pathogens, the problems we face in combating these resistant bacteria and potential effective agents for the treatment of UTIs caused by multidrug-resistant pathogens.

10. Das R, Perrelli E, Towle V, et al. Antimicrobial susceptibility of bacteria isolated from urine samples obtained from nursing home residents. *Infect Control Hosp Epidemiol.* 2009;30(11):1116–9.
11. Sundvall PD, Ulleryd P, Gunnarsson RK. Urine culture doubtful in determining etiology of diffuse symptoms among elderly individuals: a cross-sectional study of 32 nursing homes. *BMC Fam Pract.* 2011;12:36
12. George CE, Norman G, Ramana GV, Mukherjee D, Rao T. Treatment of uncomplicated symptomatic urinary tract infections: Resistance patterns and misuse of antibiotics. *J Family Med Prim Care.* 2015 Jul-Sep; 4(3): 416–421.
13. Gordon LB, Waxman MJ, Ragsdale L, Mermel LA. Overtreatment of presumed urinary tract infection in older women presenting to the emergency department. *J Am Geriatr Soc.* 2013;61:788–92

14. Sugianli AK, Ginting F, Kusumawati RL, et al. Antimicrobial resistance in uropathogens and appropriateness of empirical treatment: a population-based surveillance study in Indonesia. *Journal of Antimicrobial Chemotherapy*. 2017;72(5):1469-1477.
15. Ilić T, Gračan S, Arapović A, et al. Changes in bacterial resistance patterns in children with urinary tract infections on antimicrobial prophylaxis at University Hospital in Split. *Medl Sci Monitor* 2011;17:CR355–61
16. Edlin RS, Shapiro DJ, Hersh AL, et al. Antibiotic resistance patterns of outpatient pediatric urinary tract infections. *J Urol* 2013;190:222–7.
17. Poorabbas B, Mardaneh J, Rezaei Z, et al. Nosocomial Infections: multicenter surveillance of antimicrobial resistance profile of *Staphylococcus aureus* and Gram negative rods isolated from blood and other sterile body fluids in Iran. *Iran J Microbiol* 2015;7:127
18. Grabe M, Bartoletti R, Bjerkklund Johansen TE. et al. *Guidelines on Urological Infections*. 2015

19. Gupta K, Hooton TM, Naber KG. et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011; 52: e103–20.