Antimicrobial Resistance of E.coli compared with other Uropathogens in patients with UTI

*Zouha Zafar, **Dr.Abid Ali, **Umair Waqas, **Waqar Mehmood, *Asfa Ilyas, *Iqra Shehzadi

* University Institute of Medical Lab Technology, University of Lahore, Punjab, Pakistan
** Department of Allied Health Sciences, University of Chenab, Gujrat, Punjab, Pakistan.

ABSTRACT

BACKGROUND: The purpose of the study is to observe antimicrobial resistance of E.coli compared with other uropathogens in patients with UTI. Even though antimicrobial resistance has been increasing over the past years all over the world, and urinary tract infection is also getting common day by day. So it needs a for time to time study to determine antibiotic susceptibility and resistance of E.coli from the cases of UTI, to identify the prevalence of E. coli among other uropathogens in UTI, to find out the most susceptible drugs against different uropathogens causing UTIs.

Methodology: Data was collected according to the rule and regulations set by the ethical committee of university of Lahore. The participants for this data was selected according to the inclusion and exclusion criteria. Three hundred samples was collected from the patients with urinary tract infection symptoms without any age limit and gender discrimination from laboratories. Urine samples was taken in sterile urine culture and sensitivity container. Urine specimens was assessed by dipstick analysis and all specimens with a positive dipstick (at least one positive nitrite reaction or leukocyte reaction) was be process for bacterial culture. Then biochemical tests was applied and results was taken out.

Results: Urine test were performed to check UTIs. Total 500 sample size were tested and most of sample show no-growth, Antibiotic E.coli cause more resistance among all other antibiotics against drugs. Antibiotic E.coli cause more resistance among all other antibiotics against drugs where as other. TGC shows 2 resistance and 50 sensitive to E.coli, The SCF shows 7 resistance and 53 sensitive to E.coli, while other organisms were also involved. The frequency and percentage of both negative is higher whereas nitrate positive is lower among them all.

Conclusion(s): The results obtained in this study indicated that *E. coli* was the most prevalent uropathogen who cause UTI, being responsible for more than half of the urinary tract infections. The frequency and percentage of both nitrate and leukocyte negative is higher whereas only nitrate positive is lower among them all.

***Keywords*:** Antimicrobial Resistance, E.coli, Uropathogens, UTI.

INTRODUCTION

Urinary tract infections are the most prevalent bacterial infections in humans, and *Escherichia coli* is definitely the most common bacterial pathogen among patients with this illness in hospitals and community[1,2].Less common uro-pathogenic bacteria include Proteus mirabilis, Klebsiella spp., and Staphylococcus saprophyticus [3].

UTIs are classified as complex and non-complex according to the type of antimicrobial therapy duration given to the patient [4,5]. Non-Complex UTIs usually have an effect on children, women and aged people who do not have anatomical urinary tract abnormality. Noncomplex UTIs hardly cause serious damage, and treated by host immune system without use of antibiotic treatment [4].

Alternatively, Complex UTIs are linked with high risk of persistent infection and it call for a prolonged treatment. Complex UTIs are linked with urinary tract disorders like urinary obstruction and retention, previous antibiotic exposure, and renal failure. Together, these elements damage the urinary system, raising the possibility of severe consequences and treatment failure [6].

Not all the strains of E. coli from the intestinal tract cause urinary tract infection, instead a subgroup elected by factors which support extra intestinal survival. Such factors include use of flagella for movement, presence of fimbriae or pili in a structure and chemical adhesion[7]. The type-1 pili, which are the most prevalent uro-pathogenic E. coli strains are known to attach to mannose-containing glycoprotein receptors on facet cells lining of the bladder or vaginal epithelial cells, are known to be linked with more severe urinary tract infections (UTIs) [8]. Many virulence factors have also been found [7] that give some faecal E. coli the potential to colonize the vaginal mucosa and produce symptomatic urine illnesses. This group of *E. coli* is known collectively as uropathogenic *E. coli* (UPEC) clones [9].

Minimum one symptomatic UTI episode is thought to occur in at least 40% of women and 12% of men throughout the course of their lifetimes, and 27 to 48% of the affected women experience recurring UTIs [9, 10]. Roughly 50% of all bacterial infections that cause higher morbidity and longer hospitalization are UTIs, which account for about 40% of all hospital-acquired infections [10,11]. Every year, 150 million individuals worldwide are affected by urinary tract infections (UTIs) [12], which are among the most prevalent bacterial illnesses [13]. In 2002, the predictable number of HAI (health care acquired infection) in US hospitals was about 1.7 million. The estimated deaths associated with HAI in US hospitals were 98,987: of these, 13,088 for urinary tract infections [14].

The rationale of the study is to observe antimicrobial resistance of E.coli compared with other uropathogens in patients with UTI. Even though antimicrobial resistance has been increasing over the past years all over the world, and urinary tract infection is also getting common day by day. So it needs a for time to time study to determine antibiotic susceptibility and resistance of E.coli from the cases of UTI, to identify the prevalence of E. coli among other uropathogens in UTI, to find out the most susceptible drugs against different uropathogens causing UTIs.

Material and methods:

Study design, duration and setting

Retrospective cross-sectional study was conducted. A sample of 500 people were selected from Clinical Microbiology Section of Pak Medical Center (PMC).

Inclusion:

- Patients come with symptoms of pain or burning while urinating, frequent urination or blood in urine
- UTI Patients diagnosed by chemical urinalysis (dipstick test) which show at least one positive blood, nitrites or leukocyte.
- Age: All age groups will be included.
- Gender: Both male and female.

Exclusion:

- Exclusion Criteria include those patients who have any kind of other infection.
- Bacteria which were not identified as e.coli, klebsiella pneumoniae, Pseudomonas and Proteus will be excluded in this study.

Ethical approval and Consent

Ethical approval was taken from Institutional Review Board (IRB) of University of Lahore, Punjab, Pakistan. An informed was taken from the participants before collecting data. Ensured that data would be used for only research purpose. The research project was approved by research and ethics committee of University of Lahore

Data collection procedure and tools

The request form was design as a data collection tool in order to collect the information from the Laboratory. The form will be comprised of data of microbiology section of the Lab.

Samples collection and processing:

- Urine first morning specimen or 8-hour specimen was collected from the patients in department of microbiology.

- Three hundred samples was collected from the patients with urinary tract infection symptoms without any age limit and gender discrimination. Urine samples was taken in sterile urine culture and sensitivity container.

ANALYSIS

- Urine specimens was assessed by dipstick analysis and all specimens with a positive dipstick (at least one positive nitrite reaction or leukocyte reaction) was be process for bacterial culture.

Bacterial Identification

- Cultures was inoculate on MacConkey media and Cystine-Lactose Electrolyte-Deficient (CLED) media to obtain isolated colonies. After inoculation, media plates was incubate into an incubator at 37°C. After 24 hours of incubation, if pure growth shown it was consider for further gram staining and biochemical analysis with antimicrobial susceptibility testing.

Gram Staining

Gram stain was use to differentiate between gram-positive and negative bacteria. After confirming the bacterial nature, the biochemical tests were performed for identification and characterization of isolates along with antimicrobial testing.

Biochemical Identification

Biochemical analysis was perform to distinguish the bacterial strains. Different types of biochemical analysis include indole, methyl red, Voges-Proskauer, citrate test motility, indole, urease, citrate, and oxidase assays were used for gram-negative rods (GNRs),

AST (Antimicrobial susceptibility testing)

- Antimicrobial susceptibility testing was done. Antimicrobial susceptibility of *E. coli* was be test by the disk diffusion method according to the CLSI recommendations, using the Mueller-Hinton agar. Pure colonies was used to make inoculums. Different antibiotics were dispensed on Mueller-Hinton agar.

- The antibiotics used were ampicillin, nitrofurantoin, ciprofloxacin, sulfamethoxazole, fosfomycin, meropenem and gentamicin

- Zone diameters was be measured and results were interpreted as susceptible, intermediate or resistant according to criteria established by CLSI.[15]

Statistical analysis:

The Statistical Package for Social Sciences (SPSS) 20.0 was used to examine the data (SPSSA Inc. Chicago, USA). The categorical value was expressed in the form of frequency and percentages. The data was shown using pie charts and bar charts. To examine the data, appropriate statistical methods were used.

Results:

Five hundred samples was collected from the patients with urinary tract infection symptoms without any age limit and gender discrimination. Leukocyte and nitrate tests were applied whereas oganisms like E.coli, klebsiella Pneumonia, staphylococcus Aureus and Proteus sensitivity and resistance reaction were seen among antibiotics like Salbactum-Cefoparazone (SCF) , Amoxacillin (AMC), Tigecucline (TGC), Amikacin (AK), Sulphamhoxazole (SXT), Ciprofloxaxin (CIP), Imipenem (IPM), Gentamycin (CN), Cefepime (EP), Tazobactum (TZP), Tobramycin (TOB), Ampicillin (AMP).

Organisms sensitive and resistance cross-tabulation is described in first 3 table. One by one they are described. Table 4 describes the frequency and percent of chemical test. The frequency and percentage of both negative is higher whereas nitrate positive is lower among them all. Table 5-8 describes Chemical Test Organisms Cross tabulation. Antibiotic E.coli cause more resistance among all other antibiotics against drugs. Antibiotic E.coli cause more resistance among all other antibiotics against drugs where as other.

TABLE 1 ORGANSISMS CROSSTABULATION

		(SCF)		(AMC)		(TGC)		(AK)
		Resistant	sensitive	Resistant	sensitive	Resistant	sensitive	sensitive
Organism	E-coli	7	52	49	11	2	50	53
	Klebsiella Pneumonia	1	6	7	0	0	13	8
	Staphylococcus s Aureus	2	8	5	2	12	3	18
	Proteus	0	5	1	3	5	1	4
Total		10	71	62	16	19	67	83

TABLE 2 ORGANSISMS CROSSTABULATION

		(SXT)		(CIP)		(IPM)		(CN)	
		Resistant	sensitive	Resistant	sensitive	Resistant	sensitive	Resistant	sensitive
Organism	E-coli	9	14	25	9	0	42	20	42
	Klebsiella Pneumonia	0	2	4	1	0	8	1	12
	Staphylococcus Aureus	3	8	3	17	2	3	1	32
	Proteus	2	0	6	1	0	6	2	5
Total		14	24	38	28	2	59	24	91

TABLE 3 ORGANSISMS CROSSTABULATION

		(EP)		(TOB)		(AMP)		(TZP)	
		resistant	sensitive	resistant	sensitive	resistant	sensitive	resistant	sensitive
Organism	E-coli	0	3	9	8	49	9	1	56
	Klebsiella Pneumonia	0	0	0	3	10	0	1	12
	Staphylococcus Aureus	2	6	0	22	22	1	0	21
	Proteus	0	2	0	4	6	0	0	5
Total		2	11	9	37	87	10	2	94

TABLE 4 CHEMICAL TEST

	Frequency
Both Negative	363
Leukocyte Estrase Positive	38
Nitrate Positive	27
Both Positive	72
Total	500

Table 5

Chemical Test * Organisms Crosstabulation

Chemical Test	Organisms				Total
	No Growth	Klebsiella Pneumonia	Staphylococcus Aureus	Proteus	
Both Negative	360	1	1	1	363
Total	360	1	1	1	363

Table 6

Chemical Test * Organisms Crosstabulation

Chemical Test	Organisms					Total
	No Growth	E-coli	Klebsiella Pneumonia	Staphylococcus Aureus	Proteus	
leukocyte Positi	17	13	2	5	1	38
Total	17	13	2	5	1	38

Table 7

Chemical Test * Organisms Crosstabulation

Chemical Test	Organisms				Total
	E-coli	Klebsiella Pneumonia	Staphylococcus Aureus	Proteus	
Both Positive	39	10	19	4	72
Total	39	10	19	4	72

Table 8

Chemical Test * Organisms Crosstabulation

Chemical Test	Organisms				Total
	No Growth	E-coli	Staphylococcus Aureus	Proteus	
Nitrate Positive	1	17	8	1	27
Total	1	17	8	1	27

Discussion:

Urinary tract infections (UTIs) are the second most common infectious presentation in community medical practice. Worldwide, about 150 million people are diagnosed with UTI each year. 15 About 80% of UTIs are caused by E. coli[16]. Leukocyte and nitrate tests were applied whereas oganisms like E.coli, klebsiella Pneumonia, staphylococcus Aureus and Proteus sensitivity and resistance reaction were seen among antibiotics like Salbactum-Cefoparazone (SCF) , Amoxacillin (AMC), Tigecucline (TGC), Amikacin (AK), Sulphamhoxazole (SXT), Ciprofloxaxin (CIP), Imipenem (IPM), Gentamycin (CN), Cefepime (EP), Tazobactum (TZP), Tobramycin (TOB), Ampicillin (AMP).

In our study results major UTIs are caused by E. coli. Antibiotic E.coli cause more resistance among all other antibiotics against drugs. Other researches also shown the major cause of E.coli in UTIs. According to study it is shown that the prevalence of *E. coli* isolated from urine significantly increased in this time period and that fosfomycin was more frequently prescribed at the expense of co-amoxiclav, co-trimoxazole, and fluoroquinolones.[17]

The rise in prevalence over time of *E. coli* was a remarkable finding. Although the microbiological analysis of our last survey was performed in another laboratory, the methods were comparable. The 2009 survey showed already a significant increase in prevalence of *E. coli* compared to 2004. The prevalence of *E. coli* in UTI ranged from 76.7 to 84% in different studies. [18]

In our study results describe that other with E.coli other pathogens also affect the body and causes UTIs while other Studies from different parts of Iraq and worldwide showed that lactose fermenters E. coli and Klebsiella were the most common pathogens associated with UTIs. These results were in agreement with the results obtained from other studies conducted worldwide which approved that E. coli is the major pathogen that cause UTIs. As E. coli is a major normal flora in the gut and most of the times poor hygiene will lead to cross contamination and then urinary tract infections. [19]

Antimicrobial resistance in uropathogenic E. coli is of major concern worldwide due to its increasing resistance to several commonly prescribed antibiotics. In our study, E. coli isolates were various in their susceptibility to different antibiotics belonging to different groups[20]

Study analyzed current resistance patterns of *E. coli* isolated from patients presenting with UTIs. To distinguish between true infection and contaminations, we applied two different analyses: (i) in a broad approach, patients were included if they were diagnosed with and treated for UTI by their physician ('all patients group', reflecting clinical practice) and (ii) only patients whose symptoms typical for UTI were

documented ('symptomatic patients group'; a better reflection of true UTI). There results shows patients with a UTI diagnosed by the attending physician with *E. coli* isolated in urine culture were included.[17]

Higher prevalence of UTI among females is due to various factors that predispose women to UTI. The most prevalent urinary tract pathogen in both the genders in our study was *E. coli* and *K. pneumoniae* in concordance. Diagnosis of UTI is dependent on both the presence of clinical signs and symptoms along with a positive urine culture; however, in majority of health-care settings on the scene, this diagnosis is accomplished and treatment is started without performing the culture and antimicrobial sensitivity.[21]

CONCLUSIONS

The results obtained in this study indicated that *E. coli* was the most prevalent uropathogen who cause UTI, being responsible for more than half of the urinary tract infections. The frequency and percentage of both nitrate and leukocyte negative is higher whereas only nitrate positive is lower among them all.

Conflict of Interest: The authors declare that there is no conflict of interest regarding this study.

Funding: No funding

Data availability statement: Supported data will be provided on request from the corresponding author.

REFERENCES

1. Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *J Antimicrob Chemother* 2001; **47**(6): 773-80.
2. Kucheria R, Dasgupta P, Sacks S, Khan M, Sheerin N. Urinary tract infections: new insights into a common problem. *Postgraduate medical journal* 2005; **81**(952): 83.
3. Okarska-Napierała M, Wasilewska A, Kuchar E. Urinary tract infection in children: Diagnosis, treatment, imaging - Comparison of current guidelines. *J Pediatr Urol* 2017; **13**(6): 567-73.
4. Hooton TM. Clinical practice. Uncomplicated urinary tract infection. *N Engl J Med* 2012; **366**(11): 1028-37.
5. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol* 2015; **13**(5): 269-84.
6. Lichtenberger P, Hooton TM. Complicated urinary tract infections. *Curr Infect Dis Rep* 2008; **10**(6): 499-504.
7. Emody L, Kerényi M, Nagy G. Virulence factors of uropathogenic Escherichia coli. *Int J Antimicrob Agents* 2003; **22 Suppl 2**: 29-33.
8. Venegas MF, Navas EL, Gaffney RA, Duncan JL, Anderson BE, Schaeffer AJ. Binding of type 1-piliated Escherichia coli to vaginal mucus. *Infect Immun* 1995; **63**(2): 416-22.
9. Orskov I, Orskov F. Escherichia coli in extra-intestinal infections. *J Hyg (Lond)* 1985; **95**(3): 551-75.
10. Brumbaugh AR, Smith SN, Mobley HL. Immunization with the yersiniabactin receptor, FyuA, protects against pyelonephritis in a murine model of urinary tract infection. *Infect Immun* 2013; **81**(9): 3309-16.
11. Micali S, Isgro G, Bianchi G, Miceli N, Calapai G, Navarra M. Cranberry and recurrent cystitis: more than marketing? *Crit Rev Food Sci Nutr* 2014; **54**(8): 1063-75.
12. Asadi Karam MR, Habibi M, Bouzari S. Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against Uropathogenic Escherichia coli. *Mol Immunol* 2019; **108**: 56-67.

13. Stamm WE, Norrby SR. Urinary tract infections: disease panorama and challenges. *J Infect Dis* 2001; **183 Suppl 1**: S1-4.
14. Klevens RM, Edwards JR, Richards CL, Jr., et al. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Health Rep* 2007; **122**(2): 160-6.
15. Bonadio M, Meini M, Spitaleri P, Gigli C. Current microbiological and clinical aspects of urinary tract infections. *Eur Urol* 2001; **40**(4): 439-44; discussion 45.
16. MacKinnon M, Sargeant J, Pearl D, et al. Evaluation of the health and healthcare system burden due to antimicrobial-resistant Escherichia coli infections in humans: a systematic review and meta-analysis. *Antimicrobial Resistance & Infection Control* 2020; **9**: 1-22.
17. van Driel AA, Notermans D, Meima A, et al. Antibiotic resistance of Escherichia coli isolated from uncomplicated UTI in general practice patients over a 10-year period. *European Journal of Clinical Microbiology & Infectious Diseases* 2019; **38**: 2151-8.
18. Seitz M, Stief C, Waidelich R. Local epidemiology and resistance profiles in acute uncomplicated cystitis (AUC) in women: a prospective cohort study in an urban urological ambulatory setting. *BMC Infectious Diseases* 2017; **17**(1): 1-5.
19. Polse RF, Yousif SY, Assafi MS. Prevalence and antimicrobial susceptibility patterns of uropathogenic E. coli among people in Zakho, Iraq. *International Journal of Research in Medical Sciences* 2016; **4**(4): 1219-23.
20. Moyo SJ, Aboud S, Kasubi M, Lyamuya EF, Maselle SY. Antimicrobial resistance among producers and non-producers of extended spectrum beta-lactamases in urinary isolates at a tertiary Hospital in Tanzania. *BMC research notes* 2010; **3**(1): 1-5.
21. Ahmed SS, Shariq A, Alsalloom AA, Babikir IH, Alhomoud BN. Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *International Journal of Health Sciences* 2019; **13**(2): 48.

Authors

First Author: Zouha Zafar, DMLS, University institute of Medical Lab Technology, The University of Lahore, Punjab, Pakistan.

Second Author: Dr. Abid Ali, Head of Department of Doctor of Medical Lab Sciences, Assistant Professor in Allied Health Sciences, University of Chenab, Gujrat, Punjab, Pakistan.

Third Author: Umair Waqas, Imran Idress Institute of Rehabilitation Sciences, Sialkot.

Fourth Author: Waqar Mehmood Dar, Lecturer, The University of Chenab, Gujrat.

Fifth Author: Asfa Ilyas, the University of Chenab, Gujrat.

Sixth Author: Iqra Shehzadi, the University of Chenab, Gujrat.