ORIGINAL ARTICLE

Diversity of Uropathogens and their Antibiotic Susceptibility Pattern

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ABSTRACT

Bacterial urinary tract infection (UTI) is one of the utmost prevailing bacterial diseases that affect both sexes at different ages. Due to emerging multi-drug resistant (MDR) bacteria, it is necessary to investigate the antibiotic susceptibility pattern of the bacterial agents to improve the empirical treatment. In this study, we collected a total of 142 urine samples from infected individuals of both sexes at Al-khidmat diagnostics and blood bank from January to March 2022. Different antibiotic susceptibility patterns of the bacterial isolates were examined by using the disk diffusion method. The UTI was reported in 21 (15 %) males and 119 (85 %) females. the highest number of positive individuals are found in middle and old age groups respectively 40 % and 44 %. While 96 % of uropathogens are gram-positive and only 3 % of cases were caused by gram-negative bacteria. The most prevalent pathogen was E.coli 62 % and followed by Staphylococcus Aureus 12 %. In study showed that E.coli is 62 % amoxicillin, Cefuroxime 23, Cefixime 48 %, Norfloxacin 24, Penicillins 37 % , Ciprofloxacin 24 %, Gentamycin 8 %, Ceftriaxone 20 %, and Levofloxacin 4 % resistant. The current study concluded that antibiotic resistance among urinary pathogens was raised, and significant variance in antibiotic susceptibility patterns was identified. UTIs can be treated with medicines such as Levofloxacin, Gentamycin, Teicoplanin, and Moxifloxacin. This study's findings indicate that future research should focus on the causes of resistance to identify solutions to this crucial problem, as well as on the use of health education to prevent drug usage.

Keywords: Uropathogens, Antibiotics, Urinary tract infection, UTI, MDR

INTRODUCTION

Urinary tract infection (UTI) is a major health care concern worldwide¹. UTIs are mostly caused by the existence and growth of bacteria in the urinary tract. It could involve the inferior urinary tract or the urinary bladder. Afterward anemia and UTIs are the next common problems in expectant women².UTIs are one of the utmost prevailing bacterial diseases in. females and aging individuals³. According to the Centers for Disease Control and Prevention (CDC), UTIs are the most prevalent bacterial diseases needing medical care UTI infection is the leading cause of illness and health care disbursements in persons of all ages⁴. Women are extremely vulnerable to UTIs infection than men. They happen commonly from the years 16 to 35 ages, with more than 10 % of women suffering UTIs during their life span⁵. Single in five women will have a minimum of one UTI in her lifetime. Approximately twenty percent of females who have a UTI infection will have another, and 30% of persons will have nevertheless another. Of this last group, 80 % will have relapses. Nearby 80 to 90 % of UTIs are instigated by a single type of microorganism and that is bacteria6.

The bulk of UTIs occurs in sexually full of zip women. Threat upsurges by 3-5 intervals when diaphragms are used for contraception. UTIs infection risk also rises marginally with not voiding after sexual intercourse and usage of spermicide. Increased risk has not been established with oral contraceptives, not voiding before intercourse or the use of condoms. Non-cotton underwear. UTIs are the most common health concern worldwide, especially in developing countries. And extensive use of antibiotics alongside uropathogens has led to the rise of antibiotic-resistant. The rise of antibiotic resistance in the management of UTIs is a thoughtful public health issue. Mainly in the developing countries where there is from top to toe level of poverty, poor hygienic practices, and illiteracy⁷. Prevalence of antibiotic-resistant is also high there where the easy availability of drugs in the community without prescription and abuses of drugs⁸.

Among different pathogenic which are etiological agents for UTI, the predominant bacteria is Escherichia coli (E.Coli) while it is uncomplicated UTI infections in females, related to more than 80 % of infections⁹. Staphylococcus saprophyticus is also major bacteria approximately 15 % of cases are accounted for. Supplementary members of the Enterobacteriaceae family, such

as Proteus sp. Enterobacter sp. Klebsiella spare is associated with unfussy UTI infections. Group B streptococci are a rare pathogen in UTI infection in young healthy females but need treatment in expecting women¹⁰. UTI infection is the most common and transmissible infection. Because of its difficulties, it injures various delicate structures in the body, including the ureters, urethra, and bladder. UTIs are more common in females than in males¹¹, especially in pregnant women due to anatomical differences and a lower level of urine throughout pregnancy. Pregnancy increases bacteriuria, which leads to pyelonephritis and obstetric complications such as greater fetal death rates, low birth weight, and preterm. The causative microorganism and its virulence factors determine the mechanisms of UTI infection¹².

Prevalence and resistance of uropathogenic microorganisms against antimicrobials are increasing universally over time. Bacteria for example E.coli, Acinetobacter, and Pseudomonas aeruginosa are particularly notable due to their intrinsic capability of MDR¹³. UTIs are frequently treated by broad-spectrum antibiotics. Fluoroquinolone is a drug choice as an initial agent for the empiric treatment of UTIs in a region where resistance is probable to be a great concern. This is because they have great bacteriological and clinical therapy rates, in addition to low rates of resistance, among the most common UTIs pathogens¹⁴. The circumstances are even more puzzling with the increasing frequency of UTIs in addition to the reduced discovery of new medicine¹⁵. Uropathogenic E. coli is one of the utmost causes of urinary tract. Worldwide epidemiological research shows the incidence of MDR creating Enterobacteriaceae equally in the community-acquired and healthcare-associated infections¹⁵

Because of the multi-drug, there have been several changes in the antimicrobial susceptibility patterns of urinary tract bacteria, and because of antibiotic-resistant microorganisms, management of UTIs has become challenging, and the prevalence of infection is growing day by day. The globe, the unexpected development, as well as the rise of multidrug-resistant bacterial infections, is a major public health concern that is expanding by the day. For the past two or three years, hospital-acquired UTIs have been on the rise due to the emergence of multidrug-resistant UTI bacteria¹⁶. As a result, the current investigation sought to ascertain the prevalence of uropathogens and MDR uropathogenic bacterial strains in Rawalpindi, Pakistan.

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MATERIALS AND METHODS

This cross-sectional hospital-based study was conducted at Alkhidmat diagnostics and blood bank in Punjab, Pakistan, from January to March 2022. Patients with symptomatic UTIs range in age from 0 to 80 years. All of the patients were from the Punjab district of Rawalpindi in Pakistan. They all meet the inclusion criteria for this study. A total of 142 urine samples were obtained from infected men and women who presented to Al-khidmat diagnostics and blood banks with symptoms of UTIs.

To reduce contamination, the clean-catch technique was used to collect specimens. Each suspect's fresh and midstream urine was collected into a sterile screw-capped container. The sample was properly labeled, transported to the medical laboratory, and examined within 2 hours of being collected. All patients were instructed and guided on how to collect urine specimens aseptically to avoid contamination before specimen collection. Furthermore, an agreement was sought from all participants before specimen collection, and the laboratory received ethical approval.

Only patients who presented with clinical symptoms of UTI and a positive + urine culture were investigated, and the samples were processed accordingly. In a nutshell, each urine sample was treated aseptically within one hour. The disc was used to investigate the antibiotic susceptibility patterns of bacterial isolates. Cefuroxime, Gentamicin, Cefixime, Ciprofloxacin, Levofloxacin, Penicillins, and Teicoplanin, as well as Amoxicillin, were employed in the antibiotic disc. Standardized overnight cultures of each isolate were used to seed melting Mueller-Hinton agar (MHA) at 45 C, which was then aseptically poured into sterilized plates (in triplicate). The MHA plates were then incubated for 24 hours at 37 degrees Celsius. The unambiguous inhibitory zones were measured and analyzed after twenty-four hours of incubation.

Microsoft Excel 2020 was used to enter and evaluate the antibiotic resistance data.

RESULT

A total of 199 urine samples were collected, the frequency of UTIs were 21 (15 %) in male and 119 (85 %) in female (Table 1).

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S. No	Gender	Number of positive	Percentage %
1	Females	119	85
2	Males	21	15



Figure 1. The gender-wise distribution of UTIs infection.

In this study, the highest number of positive individuals are found in middle and old age groups respectively 40 % and 44 % and the less number of cases are found in the low age group 3-16.

Table 2. Age-wise distribution of UTIs infection.

S. No	Age Group Name	Age Ranges	Number of Positive Cases	
1	Babies	0-2	0	
2	Children's	3—16	13	
3	Young adults	17-30	43	
4	Middle aged-adults	31-45	44	
5	Old adults	Above 45	40	



Figure 2. Age-wise distribution of urinary tract infections (UTIs)

In the current study, 96 % of uropathogens are gram-positive and only 3 % of cases were caused by gram-negative bacteria.





Name of Bacteria	Number of Bacteria	Percentage %
Escherichia coli	25	62
Enterococcus spp.	3	7.5
Pseudomonas spp.	4	10
Staphylococcus aureus	5	12.5
Staphylococcus saprophyticus	3	7.5



Figure 4. Culture sensitivity to nutrient agar

In the current finding, the most prevalent pathogen is E.coli 62 % and followed by Staphylococcus Aureus 12 % Enterococcus species 7.5 % Pseudomonas species 10 % Staphylococcus Aureus 12.5 %, and S. Saprophyticus 7.5. In study showed that E.coli is 62 % amoxicillin, Cefuroxime 23, Cefixime 48 %, Norfloxacin 24, Penicillins 37 %, Ciprofloxacin 24 %, Gentamycin 8 %, Ceftriaxone 20 %, and Levofloxacin 4 % resistant. Enterococcus species 100 % amoxicillin, Penicillins 67 % Ciprofloxacin 33 %, Ceftriaxone 20 % resistant. Pseudomonas species 30 % amoxicillin, Penicillins 37 %, Ciprofloxacin 10 %, Ceftriaxone 20 %, and Levofloxacin 40 % resistant. Staphylococcus Aureus 20 % amoxicillin, Penicillins 40, Gentamycin 49 %, and Ceftriaxone 20 %, Norfloxacin 30, Penicillins 40, Ciprofloxacin 40 %, Gentamycin 40 %, and Levofloxacin 40 %, Rentamycin 40 %, Renta

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Figure 5. Bacterial culture on blood agar.

Antibiotics	Percentage (%) of bacterial sensitivity and resistance	E.coli	Enterococcus spp.	Pseudomonas spp.	Staphylococcus aureus	Staphylococcus saprophyticus
Amoxicillin	Sensitive	37.5	0	70	80	70
	Resistant	62.5	100	30	20	30
Cefuroxime	Sensitive	68	100	100	100	100
	Resistant	23	0	0	0	0
Cefixime	Sensitive	52	100	100	100	80
	Resistant	48	0	0	0	20
	Sensitive	76	100	74	100	70
Norfloxacin	Resistant	24	0	26	0	30
Penicillin	Sensitive	63	33	70	60	60
	Resistant	37	67	30	40	40
Teicoplanin	Sensitive	92	67	100	100	100
	Resistant	8	33	0	0	0
Ciproflovooin	Sensitive	76	67	90	100	60
Cipronoxacin	Resistant	24	33	10	0	40
Gentamycin	Sensitive	92	100	100	60	60
	Resistant	8	0	0	49	40
Ceftriaxone	Sensitive	80	100	80	100	100
	Resistant	20	0	0	0	0
Lovefloxeein	Sensitive	96	100	60	100	60
Levolioxacin	Resistant	4	0	40	0	40



Figure 6. Bacterial antibiotic sensitivity pattern.

DISCUSSION

A total of 199 urine samples were collected, the frequency of UTIs were 21 (15 %) in male and 119 (85%) in female. A similar study was reported by Zubair et al in 2019 in which the number of positive patients was 45% male and 55 % female, in district Kohat¹⁷. These results are supported by a study reported on the Frequency of UTI infection, and the sensitivity pattern of different uropathogens in diabetes¹⁷. In a similar study reported by Ullah et al gram-positive bacteria, 79.3 % were Gram-negative bacteria¹⁶.

A study reported in the Kohat region of Pakistan also reported Escherichia coli is a prevalent uropathogens 41.4 % Klebsiella pneumonia 9 15.5 % and Proteus mirabilis 8 13.8 %¹⁶. Another study reported by Zubair et al., 2019 showed that E.coli was the most frequent among individuals 71 %, followed by Klebsiella 7.48 % and Staphylococcus aureus 9.35 %¹⁷. Similarly, Gradwohl et al reported E.coli 75-95 % of the most common pathogen UTIs are E. coli (75%–95%¹⁰). The current finding contrast with a study that reported that the most prevalent pathogen is S. aureus 28%, E. coli 24.6%, and S. saprophyticus at 20%¹⁸.

The findings of this study will assist clinicians in prescription suitable antibiotics for the treatment of urinary tract infections. Second, the rise of MDR organisms, as we described in our study, poses a challenge to the therapy of UTI patients. To avoid the establishment of multidrug resistance, the physician should rigorously adhere to the culture report before beginning medication. We are losing antibiotics of choice for treating basic bacterial infections as a result of the evolution of multidrug resistance.

CONCLUSION

Urinary tract infections are a prevalent kind of infection. The most common bacteria are E.coli, and antibiotic resistance has recently emerged among urine isolates. The current investigation found that antibiotic resistance among urinary infections had grown, with significant Al-khidmat diagnostics and blood bank diversity in the antibiotic susceptibility pattern. UTIs can be treated with medicines such as Levofloxacin, Gentamycin, Teicoplanin, and Ciprofloxacin. This study's findings indicate that future research should focus on the reasons for resistance to identifying solutions to this crucial problem, as well as on the use of health education to prevent drug usage.

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REFERENCES

- Lawani EU, Alade T, Oyelaran D. Urinary tract infection amongst pregnant women in Amassoma, Southern Nigeria. African journal of microbiology research. 2015;9(6):355-9.
- Ranjan A, Sridhar STK, Matta N, Chokkakula S, Ansari RK. Prevalence of UTI among pregnant women and its complications in newborns. Indian Journal of Pharmacy Practice. 2017;10(1).
- Fazly Bazzaz BS, Darvishi Fork S, Ahmadi R, Khameneh B. Deep insights into urinary tract infections and effective natural remedies. African Journal of Urology. 2021;27(1):1-13.
- Al-Shami SA, Jawad AH, Jamil QT, Hamza RR, editors. The Effect of Some Factors on Virulence of E. coli Bacteria Isolated from UTI Infection.(Review study). IOP Conference Series: Earth and Environmental Science; 2021: IOP Publishing.
- Almalki MA, Varghese R. Prevalence of catheter associated biofilm producing bacteria and their antibiotic sensitivity pattern. Journal of King Saud University-Science. 2020;32(2):1427-33.
- King Saud University-Science. 2020;32(2):1427-33.
 de Vasconcelos-Pereira EF, Figueiró-Filho EA, de Oliveira VM, Fernandes ACO, de Moura Fé CS, Coelho LR, et al. Urinary tract infection in high risk pregnant women. infection. 2013;7(25):27-30.
- 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(1):1-9.

- 8. Weekes LM. Antibiotic resistance changing management of urinary tract infections in aged care. Med J Aust. 2015;203(9):352.
- Yongzhi L, Shi Y, Jia L, Yili L, Xingwang Z, Xue G. Risk factors for urinary tract infection in patients with urolithiasis—primary report of a single center cohort. BMC urology. 2018;18(1):1-6.
- Gradwohl S, Bettcher C, Chenoweth C, Van Harrison R, Zoschnick L. Guidelines for Clinical Care Ambulatory Urinary Tract Infection. University of Michigan. 2016.
- 11. Muhammad A, Khan S, Ali N, Rehman M, Ali I. Prevalence and antibiotic susceptibility pattern of uropathogens in outpatients at a tertiary care hospital. New Microbes and new infections. 2020;36:100716.
- 12. Bien J, Sokolova O, Bozko P. Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage. International journal of nephrology. 2012;2012.
- Asmat U, Mumtaz MZ, Malik A. Rising prevalence of multidrugresistant uropathogenic bacteria from urinary tract infections in pregnant women. Journal of Taibah University Medical Sciences. 2021;16(1):102-11.
- De Francesco MA, Ravizzola G, Peroni L, Negrini R, Manca N. Urinary tract infections in Brescia, Italy: etiology of uropathogens and antimicrobial resistance of common uropathogens. Medical science monitor. 2007;13(6):BR136-BR44.
- Ali I, Rafaque Z, Ahmed S, Malik S, Dasti JI. Prevalence of multi-drug resistant uropathogenic Escherichia coli in Potohar region of Pakistan. Asian Pacific Journal of Tropical Biomedicine. 2016;6(1):60-6.
- Ullah A, Shah S, Almugadam B, Sadiqui S. Prevalence of symptomatic urinary tract infections and antimicrobial susceptibility patterns of isolated uropathogens in kohat region of Pakistan. MOJ Biol Med. 2018;3(4):85-9.
- 17. Zubair KU, Shah AH, Fawwad A, Sabir R, Butt A. Frequency of urinary tract infection and antibiotic sensitivity of uropathogens in patients with diabetes. Pakistan Journal of Medical Sciences. 2019;35(6):1664.
- Ekwealor PA, Ugwu MC, Ezeobi I, Amalukwe G, Ugwu BC, Okezie U, et al. Antimicrobial evaluation of bacterial isolates from urine specimen of patients with complaints of urinary tract infections in Awka, Nigeria. International journal of microbiology. 2016;2016.