Iranian Journal of Microbiology
Volume 16 Number 4 (August 2024) 477-483

ORIGINAL ARTICLE

Precision medicine in practice: unravelling the prevalence and antibiograms of urine cultures for informed decision making in federal tertiary care- a guide to empirical antibiotics therapy

Umme Farwa[1], Samia Wazir[2], Farhan Kursheed[3*], Bisma Shoaib[4], Sheza Batool[5], Muhammad Shafiq[2]

[1]Department of Microbiology, Fazaia Medical College, Air University, Islamabad, Pakistan
[2]Department of Microbiology, Pakistan Institute of Medical Sciences, Islamabad, Pakistan
[3]Department of Microbiology, Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University, Rawalpindi, Pakistan
[4]Department of Pathology, Shifa College of Medicine, Shifa Tameer-e-Millat University, Islamabad, Pakistan
[5]Department of Pathology, Islamic International Medical College, Riphah International University, Islamabad, Pakistan

Received: April 2024**, Accepted:** July 2024

ABSTRACT

Background and Objectives: Urinary tract infections (UTIs), one of the most prevalent bacterial infections, are facing limited treatment options due to escalating concern of antibiotic resistance. Urine cultures significantly help in identification of etiological agents responsible for these infections. Assessment of antibiotic susceptibility patterns of these bacteria aids in tackling the emerging concern of antibiotic resistance and establishment of empirical therapy guidelines. Our aim was to determine various agents responsible for urinary tract infections and to assess their antibiotic susceptibility patterns.
Materials and Methods: This cross-sectional study was performed over a period of six months from January 2023 to July 2023 in Department of Microbiology of Pakistan Institute of Medical Sciences (PIMS).
Results: Out of 2957 positive samples, Gram negative bacteria were the most prevalent in 1939 (65.6%) samples followed by Gram positive bacteria in 418 (14.1%) and *Candida* spp. in 269 (9.1%) samples. In gram negative bacteria, *Escherichia coli (E. coli)* was the most prevalent bacteria isolated from 1070 samples (55.2%) followed by *Klebsiella pneumoniae* in 397 samples (20.5%). In Gram positive bacteria, *Enterococcus* spp. was the most common bacteria in 213 samples (51%) followed by *Staphylococcus aureus* in 120 samples (28.7%). Amikacin was the most sensitive drug (91%) for Gram negative bacteria. Gram positive bacteria were most susceptible to linezolid (97%-100%).
Conclusion: The generation of a hospital tailored antibiogram is essential for the effective management of infections and countering antibiotic resistance. By adopting antimicrobial stewardship strategies by deeper understanding of sensitivity patterns, we can effectively combat antibiotic resistance.

Keywords: Bacterial sensitivity test; Antibiotic susceptibility testing; Antibiotic resistance; Antimicrobial stewardship; Antimicrobial drug resistance

*Corresponding author: Farhan Kursheed, BSc, Department of Microbiology, Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University, Rawalpindi, Pakistan. Tel: +923150789926 Fax: +51-9260158 Email: farhankursheed@outlook.com

INTRODUCTION

Urinary tract infections (UTI) stand as one of the most widespread bacterial infections affecting millions of individuals worldwide, particularly women, resulting in acute morbidity, mortality and substantial healthcare costs. Every year, approximately 150 million UTI cases are diagnosed worldwide, costing more than six billion dollars in medical expenses (1). According to an estimate, roughly 50% of women suffer from UTI at least once in their lifetime (2). These infections predominantly affect individuals aged 16-64 (3). Numerous factors, including age, poor hygiene, hospitalisation, low socioeconomic status, prolonged catheterisation, sexual activity, and diabetes, are associated with the occurrence of uro-pathogens (4, 5). UTIs are primarily caused by various bacterial pathogens encompassing both gram negative and gram positive bacteria.

Antibiotic therapy has been traditionally an effective way to treat urinary tract infections (UTIs). However, the evolution and dissemination of antibiotic resistance have compromised the effectiveness of these antibiotics, posing a significant challenge in treating these infections. The emergence and dissemination of antibiotic-resistant bacteria have significantly reduced the treatment options available for infectious diseases, resulting in higher rates of morbidity, mortality, and healthcare costs globally. Antimicrobial resistance is estimated to kill approximately 1.27 million people annually, which is expected to increase to 10 million by 2050 (6).

The escalated overuse and misuse of antibiotics over the course of time has sped up the development of antibiotic resistance, leading to significantly diminished efficacy of currently available antibiotics. This situation requires immediate action to safeguard the effectiveness of antibiotics before their potency is further compromised (7).

To slow the rise of antibiotic resistance, it is crucial to assess the antibiotic susceptibility profile of etiological agents responsible for the infection, ensuring the administration of proper antibiotics in optimal dosage for effective treatment (8).

Urine is an essential clinical specimen containing various Gram positive and Gram negative bacteria, which frequently cause urinary tract infections. These bacteria include *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus* and *Enterobacter* spp. (9).

Assessing the susceptibility of antibiotics is crucial for clinicians to determine the most suitable and potent antibiotic to treat a bacterial infection. This process involves identifying bacterial response to various antibiotics, which is essential in tailoring empirical therapy and preventing the proliferation of multidrug-resistant bacterial strains (10).

The study aimed to isolate the micro-organisms present in the urine cultures and assess their antibiotic susceptibility profile to generate an antibiogram tailored to the Pakistan Institute of Medical Sciences (PIMS). This will help clinicians in decision-making and formulating essential guidelines for empirical antibiotic therapy.

MATERIALS AND METHODS

This descriptive cross-sectional study was carried out over the course of 6 months from January 2023 to June 2023 at the Microbiology Laboratory, Pathology Department of PIMS. Consecutive and non-probability sample design was used. During this time, 5,537 samples were received for culturing and antimicrobial susceptibility testing.

Patients' demographic information was gathered using pre-established Performa.

Inclusion criteria. All patients of both genders from ages 15 to 80 were included.

Exclusion criteria. This study excluded all the duplicate samples and samples from antibiotic taking patients.

Procedure. Routine Examination of urine samples was performed and pus cell were noted. The urine samples obtained were processed using the conventional aerobic bacteria culture techniques. Inoculation of urine in the laboratory was carried out as soon as possible. If some delay was expected in processing, the specimens were refrigerated. Blood agar and Cysteine Lactose Electrolyte-Deficient agar (CLED) agar media were used for inoculation. Culture of urine was carried out using the semi-quantitative strip method (MAST Bacteruritest) on CLED. After the inoculation, the culture plate was incubated aerobically at 37^{o}C for 24 hrs. In case significant growth (> 105 CFU/ml i.e., 20 CFU) was obtained, identi-

fication of the microorganisms was performed with the help of colony appearance, gram staining, catalase production, coagulase test, oxidase test, motility, biochemical profile and serology if required. API-20E (BioMerieux, France) were utilized for the biochemical identification. Kirby Baur's disk diffusion method was used to introduce the antibiotics.

Antimicrobials for Gram negatives included in the routine susceptibility testing were ampicillin (10 µg), amoxicillin/clavulanate (30 µg), piperacillin/tazobactam (110 µg), cefoperazone + sulbactam (105 µg), cefepime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin/levofloxacin (5 µg), Co-trimoxazole (25 µg), gentamicin/ amikacin (30 µg), imipenem/ meropenum (10 µg) doxycycline (30 µg), nitrofurantoin (300 µg), and Fosfomycin (200 µg).

For Gram positive bacteria, penicillin (10 µg), ampicillin (10 µg), cefoxitin (30 µg), ciprofloxacin/levofloxacin (5 µg), co-trimoxazole (25 µg), doxycycline (30 µg), vancomycin (30 µg), linezolid (30 µg), fosfomycin (200 µg) and nitrofurantoin (300 µg) were applied. The confirmation of methicillin-resistant *Staphylococcus aureus* presence was established through the agar disk diffusion technique using a 30 µg cefoxitin disc, following the guidelines of CLSI. Incubation was done at 37°C for 16-18 hours. CLSI guidelines were followed to deduce the results (11). *S. aureus* (ATCC25923), *Enterococcus faecalis* (ATCC51209), *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC27853) were used as control strains.

Analysis. The data obtained was analyzed using SPSS 28, for qualitative factors like gender, percentages and frequencies were calculated. The percentage of sensitivity of different bacterial isolates against various antibiotics was calculated. For quantitative variables like age, mean and standard deviation were computed. A value of $p < 0.05$ was taken significant.

Ethical approval. All ethical considerations were fully taken into account. The research was carried out after obtaining the approval from Ethics Research Review Board of Pakistan Institute of Medical Sciences.

RESULTS

Out of total 5,537 samples collected, positive growth was observed in 2,957 (53.4%) samples while no growth was observed in the other 2580 (46.6%) samples (Fig. 1).

Gender wise distribution of positive samples revealed that 1862 (63%) of positive samples were collected from female while 1095 (37%) were collected from male with a ratio 1.7: 1. (Fig. 2).

Majority of the samples belonged from age group 36-45 (26.6%) followed by age group 46-55 (20.3%) and 26-35 (13.5%). Mean age was calculated to be 40 + 22 years. P value was found to be insignificant (Table 1).

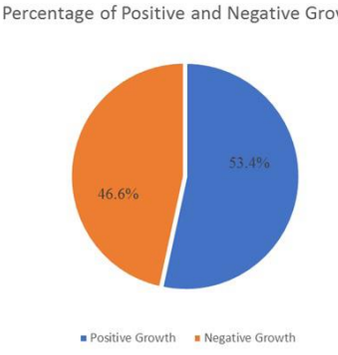

Fig. 1. Percentage of positive and negative growth in urine isolates (n=5,537)

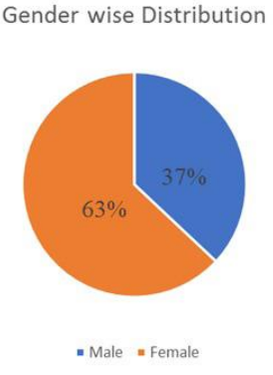

Fig. 2. Gender wise distribution of positive urine isolates (n=2957)

Table 1. Age wise distribution of positive urine isolates (n=2,957) (53.4%)

Age group	No. of patients (%)
15-25	197 (6.6%)
26-35	398 (13.5%)
36-45	788 (26.6%)
46-55	597 (20.3%)
56-65	390 (13.2%)
>65	587 (19.8%)

Among the 2,957 positive samples, 1939 (65.6%) samples had Gram negative bacteria, 418 (14.1%) samples had gram positive bacteria, and 269 samples (9.1%) showed growth of *Candida* spp. while mixed growth was observed in 331 samples (11.2%) (Table 2).

Frequency and percentages of 418 (14.1%) gram positive bacteria and 1939 (65.6%) gram negative bacteria were shown in Tables 3 and 4 respectively.

Table 2. Frequency of pathogens in positive urine isolates (n=2,957)

Type of pathogen	Number (%)
Gram Negative Bacteria	1939 (65.6%)
Gram Positive Bacteria	418 (14.1%)
Mixed Growth	331 (11.2%)
Candida spp.	269 (9.1%)

Table 3. Frequency of gram positive bacteria in positive urine isolates (n=418) (14.1%)

Name	Number
Enterococcus spp.	213 (51%)
Methicillin Susceptible S*taphylococcus aureus*	120 (28.7%)
Methicillin Resistant *Staphylococcus aureus*	85 (20.3%)

Table 4. Frequency of Gram negative bacteria in positive urine isolates (n=1939) (65.6%)

Name	Number
Escherichia coli	1070 (55.2%)
Klebsiella pneumoniae	397 (20.5%)
Proteus spp.	247 (12.7%)
Pseudomonas aeruginosa	166 (8.6%)
Enterobacter spp.	59 (3%)

The antibiotic sensitivity profile of gram negative bacteria is shown in Table 5.

The antibiotic susceptibility profile of gram positive bacteria is shown in Table 6.

DISCUSSION

Bacterial urinary tract infections are one of the major worldwide health concerns. Effective treatment of individuals with bacterial UTIs relies primarily on the identification of etiological agents of infection and selection of the most appropriate antibiotic for the treatment. This study provides substantial data regarding the most prevalent bacterial pathogens isolated from urine, along with their overall antibiotic susceptibility profile. The findings advance our knowledge regarding the most effective antibiotics to use in the treatment of bacterial illnesses.

The study revealed that females showed more infection rate than males with a ratio of 1.7 : 1 which is slightly greater than a study conducted by Khan S. et al. (2021) who found it 1.5: 1 (12). This high rate of infection in women can be attributed to their anatomy of urinary and reproductive system.

The majority of the positive samples belonged to the elderly age group >45 years old (53.3%) which corresponds with a study conducted by Akhtar N, et al. (2017) who found this percentage to be 44.6% (13).

In this study, *E. coli* was found to be the most common Gram negative pathogen in urine in 55.2% samples. Several other studies conducted in Pakistan have confirmed this prevalence of *E. coli* (14, 15). *Klebsiella pneumoniae* was the second major gram negative pathogen (20.5%) isolated from urine samples. The results are consistent with the research carried out by Al Wutayd, O. et al. (2018) (15).

Among Gram positive bacteria, *Enterococcus* spp. was the most prevalent pathogen (51%). These results are in agreement with a study carried out by Kot, B. et al. (2021) who discovered 50% of gram positive bacteria were *Enterococcus* spp. (16). MRSA was also isolated in 20.3% samples.

Besides bacterial isolates, urine samples also showed growth of *Candida* spp. (9.1%) which is higher than the study conducted by Sierra-Díaz, E., et al. (2019) who discovered this frequency to be 3.34% (17). This suggests that the prevalence of various isolates might vary depending upon time, place & how stringent infection control practices were where the research was conducted.

This study showed that all gram negative bacteria are highly resistant to Ampicillin. This resistance has been shown in various studies conducted worldwide. In our study, Amikacin was the most effective drug (91%) against gram negative Bacteria like *E. coli, K. pneumoniae, Enterobacter* spp. and *Proteus* spp. These findings agree with the results of a study performed by Angoti, G., et al. (2016) who found the susceptibility of amikacin to be 95%. (18). However, the results are not comparable with the study performed

Table 5. Antimicrobial sensitivity % of gram negative bacteria isolated from urine (n= 1939)

Antibiotic	*E. coli*	*Enterobacter* spp.	*K. pneumonia*	*Proteus* spp.	*P. aeruginosa*
Ampicillin	10%	10%	IR	IR	IR
Amox-clavulanate	15%	14%	21%	58%	IR
Pipra-tazobactam	58%	58%	40%	90%	65%
Cefoperazone-sulbactam	58%	57%	50%	89%	64%
Cefipime	20%	22%	25%	NT	40%
Ceftriaxone	28%	26%	25%	68%	IR
Ceftazidime	NT	NT	NT	NT	38%
Ciprofloxacin	20%	19%	22%	45%	67%
Levofloxacin	20%	20%	21%	50%	60%
Co-trimoxazole	28%	26%	38%	25%	IR
Gentamicin	72%	70%	50%	73%	63%
Amikacin	94%	95%	80%	95%	81%
Imipenam	87%	88%	55%	95%	60%
Meropenam	92%	93%	55%	98%	64%
Doxycycline	36%	40%	50%	IR	IR
Nitrofurantoin	93%	95%	41%	IR	NT
Fosfomycin	96%	NT	NT	NT	NT

NT: the isolate has not been tested against the drug since the result is not validated by latest CLSI testing protocols.
IR: Intrinsically Resistant

Table 6. Antimicrobial sensitivity % of gram positive bacteria isolated from urine (n= 418)

Antibiotic	*S. aureus*	MRSA	*Enterococcus* spp.
Penicillin	4%	0	61%
Ampicillin	4%	0	61%
Cefoxitin	100%	0	NT
Ciprofloxacin	30%	19%	37%
Levofloxacin	40%	20%	48%
Co-trimoxazole	60%	40%	IR
Doxycycline	90%	70%	40%
Vancomycin	100%	100%	96%
Linezolid	100%	97%	100%
Fosfomycin	NT	NT	90%
Nitrofurantoin	88%	85%	71%

by A. A. J. Aljanaby (2019) who found Imipenem to be the most sensitive drug for gram Negative bacteria (98.8%) (19). It should be taken into consideration that the susceptibility patterns of bacteria vary from time to time and place to place.

In gram positive bacteria, linezolid was the most effective drug against *Enterococcus* spp. 97%-100% which is in correspondence with a study performed by Parameswarappa, J., et al. (2013) who found the susceptibility to be 98%. (20). Among these *Enterococcus* spp., 4% Enterococci exhibited resistance to vancomycin, commonly known as Vancomycin Resistant Enterococcus (VRE). These VREs pose a significant challenge in treatment due to presence of this resistance. They can be transmitted via contaminated surfaces from one patient to another. Disinfection protocols need to be enhanced especially for rooms likely to be contaminated with VREs for the effective prevention of these infections (21). MRSA was most susceptible to vancomycin (100%) which is in correspondence with a study performed by Sonavane, A., et al. (2008) (22). UTIs are usually caused by *E. coli* from the intestines, but MRSA is increasingly causing bladder infections. This is likely due to more people carrying MRSA, and those with active infections in the groin or buttocks can spread the bacteria to the urethra. Bladder infections are also reported after lower abdominal surgeries. People with urinary catheters, like those in hospitals or with bladder control issues, are at a higher risk of MRSA-related UTIs. Preventing infection is crucial in these situations.

Pseudomonas aeruginosa is intrinsically resistant to multiple drugs including ampicillin, amox-cluvalanate, ceftriaxone, co-trimaxazole, doxycycline and nitrofurantoin. In our study, *P. aeruginosa* was most

susceptible to amikacin (81%) which is in agreement with the results of a study performed by Shah, D. A., et al. (2015) who found this susceptibility to be 74.7% (23).

Multiple factors may influence the susceptibility patterns of bacteria including location, lifestyle and disease prevention and control practices. The growing problem of antibiotic resistance must be addressed immediately by antibiotic stewardship. Our study highlights the critical need to develop and implement efficient antibiotic stewardship initiatives, such as suitable prescribing procedures, public awareness campaigns, and surveillance systems.

CONCLUSION

This study emphasises how important it is to keep an eye on the trends of antibiotic susceptibility and to understand the concerning prevalence of Multi-Drug Resistant bacteria in urine samples. Therefore, in order to apply the empirical guidelines and antimicrobial stewardship, each healthcare centre needs to create its own local antibiogram. The development of antibiotic resistance can be prevented and the effectiveness of currently available antibiotics can be preserved by improving our understanding of the antibiotic sensitivity patterns of bacteria.

ACKNOWLEDGEMENTS

The Pakistan Institute of Medical Sciences (PIMS) is truly appreciated for the vital support in making this research article possible.

REFERENCES

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol* 2015; 13: 269-284.
2. Pezeshki Najafabadi M, Dagoohian A, Rajaie S, Zarkesh-Esfahani SH, Edalati M. Common microbial causes of significant bacteriuria and their antibiotic resistance pattern in the Isfahan Province of Iran. *J Chemother* 2018; 30: 348-353.
3. Tandogdu Z, Wagenlehner FM. Global epidemiology of urinary tract infections. *Curr Opin Infect Dis* 2016; 29: 73-79.
4. Guclu E, Halis F, Kose E, Ogutlu A, Karabay O. Risk factors of multidrug-resistant bacteria in community-acquired urinary tract infections. *Afr Health Sci* 2021; 21: 214-219.
5. Choe H-S, Lee S-J, Cho Y-H, Çek M, Tandoğdu Z, Wagenlehner F, et al. Aspects of urinary tract infections and antimicrobial resistance in hospitalized urology patients in Asia: 10-Year results of the Global Prevalence Study of Infections in Urology (GPIU). *J Infect Chemother* 2018; 24: 278-283.
6. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022; 399: 629-655.
7. English BK, Gaur AH. The use and abuse of antibiotics and the development of antibiotic resistance. *Adv Exp Med Biol* 2010; 659: 73-82.
8. Khan ZA, Siddiqui MF, Park S. Current and emerging methods of antibiotic susceptibility testing. *Diagnostics (Basel)* 2019; 9: 49.
9. Amin M, Mehdinejad M, Pourdangchi Z. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. *Jundishapur J Microbiol* 2009; 2: 118-123.
10. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis* 2009; 49: 1749-1755.
11. Garcia LS. Clinical microbiology procedures handbook: American Society for Microbiology Press; 2010. https://www.google.com.pk/books/edition/Clinical_Microbiology_Procedures_Handboo/Fu2rBAAAQBAJ?hl=en&gbpv=0
12. Khan S, Moon S, Rasheed N, Hassan N, Farooq M, Akhtar T, et al. Antibiotic susceptibility profile of *E. coli* isolated from urine pus of Leady reading hospital Peshawar Pakistan. *Ann Rom Soc Cell Biol* 2021; 25: 1770-1777.
13. Akhtar N, Rahman R, Sultana S, Rahman MR. Antimicrobial sensitivity pattern of bacterial pathogens associated with urinary tract infection. *Delta Med Col J* 2017; 5: 57-62.
14. Kidwai SS, Nageen A, Ghaznavi S, Bashir F, Ara J. Antibiotic susceptibility in commonly isolated pathogens from urinary tract infection in a cohort of subjects from low socioeconomic strata. *Pak J Med Sci* 2017; 33: 254-259.
15. Al Wutayd O, Al Nafeesah A, Adam I, Babikir I. The antibiotic susceptibility patterns of uropathogens isolated in Qassim, Saudi Arabia. *J Infect Dev Ctries* 2018; 12: 946-952.
16. Kot B, Grużewska A, Szweda P, Wicha J, Parulska U. Antibiotic resistance of uropathogens isolated from patients hospitalized in district hospital in central Poland in 2020. *Antibiotics (Basel)* 2021; 10: 447.

17. Sierra-Díaz E, Hernández-Ríos CJ, Bravo-Cuellar A. Antibiotic resistance: Microbiological profile of urinary tract infections in Mexico. *Cir Cir* 2019; 87: 176-182.
18. Angoti G, Goudarzi H, Hajizadeh M, Tabatabaii Z. Bacteria isolated from urinary tract infection among patients and determination of the antibiotic susceptibility patterns of the gram negative bacteria in Iran. *Nov Biomed* 2016; 4: 1-4.
19. Majeed HT, Aljanaby AAJ. Antibiotic susceptibility patterns and prevalence of some extended spectrum beta-lactamases genes in gram-negative bacteria isolated from patients infected with urinary tract infections in Al-Najaf City, Iraq. *Avicenna J Med Biotechnol* 2019; 11: 192-201.
20. Parameswarappa J, Basavaraj VP, Basavaraj CM. Isolation, identification, and antibiogram of enterococci isolated from patients with urinary tract infection. *Ann Afr Med* 2013; 12: 176-181.
21. Correa-Martinez CL, Tönnies H, Froböse NJ, Mellmann A, Kampmeier S. Transmission of vancomycin-resistant enterococci in the hospital setting: Uncovering the patient–environment interplay. *Microorganisms* 2020; 8: 203.
22. Sonavane A, Mathur M, Turbadkar D, Baradkar V. Antimicrobial susceptibility pattern in urinary bacterial isolates. *Bombay Hosp J* 2008; 50: 240-244.
23. Shah DA, Wasim S, Essa Abdullah F. Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from urine samples of Urinary Tract Infections patients in Karachi, Pakistan. *Pak J Med Sci* 2015; 31: 341-345.