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BACTERIOLOGICAL SPECTRUM AND ANTIMICROBIAL RESISTANCE OF URINARY ISOLATES IN A TERTIARY CARE HOSPITAL

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ABSTRACT Urinary tract infections are prevalent worldwide, with Escherichia coli as the primary pathogen. The rise of antimicrobial resistance, particularly from ESBL and carbapenemase-producing strains, complicates treatment and necessitates continuous regional surveillance to guide empirical therapy. This six-month retrospective study, conducted at a tertiary care hospital in Himachal Pradesh, analyzed urine samples processed within two hours of collection. Standard culture techniques and biochemical tests identified pathogens, while antimicrobial susceptibility was assessed using the Kirby-Bauer disc diffusion method per CLSI guidelines. Out of 1,618 urine samples, 25.6% showed significant microbial growth. Escherichia coli was the most prevalent pathogen (36.4%), followed by Staphylococcus aureus (17.3%) and Enterococcus spp. (16.1%). E. coli exhibited high sensitivity to nitrofurantoin (96.7%) and fosfomycin (96%), while showing significant resistance to cefazoline (61.5%) and clotrimazole (51%).The study highlights E. coli as the leading uropathogen with notable resistance patterns. The high sensitivity to nitrofurantoin and fosfomycin supports their use as effective empirical treatments. Continuous surveillance of antimicrobial resistance patterns is crucial to inform treatment strategies and combat the growing threat of resistance. Rising antimicrobial resistance among uropathogens underscores the need for vigilant stewardship and region-specific empirical therapy to ensure effective UTI management.

KEYWORDS : Urinary Tract Infections, Uropathogens, Escherichia coli, Antimicrobial Resistance

INTRODUCTION

Urinary tract infections (UTIs) represent one of the most prevalent bacterial infections globally, affecting millions of individuals each year and imposing a substantial burden on healthcare systems.[1] These infections arise when microorganisms, primarily bacteria, colonize and multiply within the urinary tract, leading to inflammation and a variety of clinical symptoms. UTIs can affect any part of the urinary system, including the urethra, bladder, ureters, and kidneys. They range in severity from mild, self-limiting conditions to serious infections with potential for long-term health complications.[2]

Common clinical manifestations of UTIs include dysuria, increased urinary frequency ,urgency, suprapubic pain, and systemic symptoms such as fever and malaise in more advanced cases. Infections involving the upper urinary tract, such as pyelonephritis, may present with flank pain, high-grade fever, and vomiting, and carry a higher risk of complications including renal scarring. If not properly treated, UTIs can lead to permanent kidney damage, hypertension, and in severe cases, chronic kidney disease.[3]

The prevalence of UTIs is notably higher in females compared to males. This can be attributed to anatomical difference such as urethra is shorter , situated closer to the anus, facilitating the entry of bacteria from the gastrointestinal tract into the urinary system. Certain populations including pregnant women, the elderly, individuals with diabetes, those with indwelling urinary catheters, and immuno-compromised patients are at heightened risk of recurrent or complicated infections.[4]

Escherichia coli remains the most frequently isolated organism, particularly in uncomplicated cases. Other significant uropathogens include Klebsiella pneumoniae, Proteus species, Pseudomonas aeruginosa, Enterobacter species, Enterococcus species, and Staphylococcus species. The distribution and frequency of these pathogens can vary based on many factors.[5]

Effective management of UTIs relies heavily on the timely administration of appropriate antimicrobial therapy. The reliance on empirical therapy, combined with widespread misuse and overuse of antibiotics, has significantly contributed to the alarming rise in antimicrobial resistance (AMR). Resistant strains, including those producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases, are becoming increasingly common, complicating treatment protocols and limiting available therapeutic options and thus leading to longer hospital stays, higher healthcare costs, and greater morbidity and mortality rates.[6,7]

Given the dynamic nature of microbial resistance and the regional variability in pathogen distribution, continuous and comprehensive surveillance of uropathogen sensitivity patterns is essential. Such efforts are critical for informing empirical treatment guidelines, optimizing antibiotic stewardship programs, and ultimately reducing the burden of AMR on public health systems.

In view of these concerns, the present study was undertaken to identify the predominant microorganisms responsible for urinary tract infections in the study population and to assess their current patterns of antimicrobial resistance. By analyzing local susceptibility data, the study aims to provide region-specific insights that can guide more accurate and effective empirical therapy, reduce inappropriate antibiotic use, and support strategies to combat the ongoing AMR crisis.

MATERIAL & METHODS

This retrospective study was conducted in the Department of Microbiology at tertiary care hospital of Himachal for a period of six months from September of 2022 to February 2023. All the samples received in laboratory within two hours of collection were processed.

Culture was done by semiquantitative method. The inoculation was done on 5% sheep Blood agar and MacConkey agar plates by a sterile standard nichrome loop of 28 SWG, having internal diameter of loop 3.26 mm using surface streaking method. This standard loop holds 0.004 ml. The plates were incubated at 37°C for 24 hours. If no growth obtained after 24 hours, the plates were further incubated for the next

24 hours . The colony morphology on MacConkey agar and Blood agar were studied. The growth was interpreted as sterile if no growth significant if confluent growth or the number of colonies corresponded to 105 colony forming units(CFU) per milliliter (i.e . 400 colonies or more). The growth of three or more different organisms is considered as contaminates.

Identification and Biochemical Characterization of Pathogens

The bacterial isolates obtained from urine samples were identified using standard microbiological procedures, including culture characteristics and a series of biochemical tests. Identification involved the application of the catalase test (using 3% hydrogen peroxide), coagulase test, bile esculin hydrolysis, and the oxidase test. Additional biochemical analyses included the indole test citrate utilization, urease activity and the triple sugar iron (TSI) agar test.

After isolating and identifying the organism, antimicrobial susceptibility testing was done using Kirby-Bauer disc diffusion technique on Muller Hinton Agar media as per CLSI guidelines. The sizes of inhibition zones were interpreted and reported as susceptible, intermediate, or resistant.

Statistical Analysis:

The data was entered and analyzed using Excel (2021). The culture results from the collected samples were used to calculate the prevalence of UTI, characterize microbial growth (isolates), and assess antimicrobial susceptibility.

RESULTS

Out of the 1,618 urine samples, 415 (25.6%) showed significant growth, with 31.6% from males and 68.4% from females. (Figure 1)

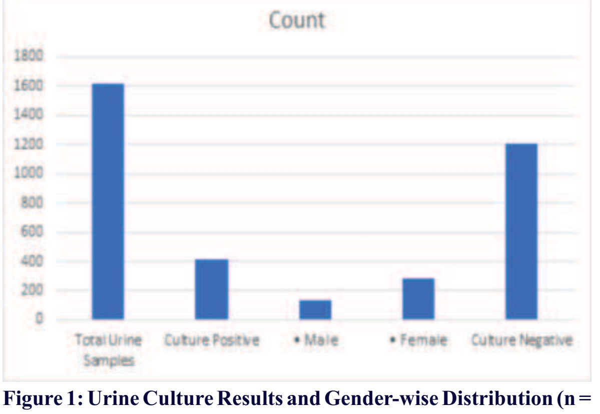

Figure 1: Urine Culture Results and Gender-wise Distribution (n = 1618)

Organisms Isolated from Positive Cultures

Among all positive samples, 34.2% were Gram-positive organisms, 59.5% were Gram-negative, and 6.3% were fungal isolates. (Figure 2)

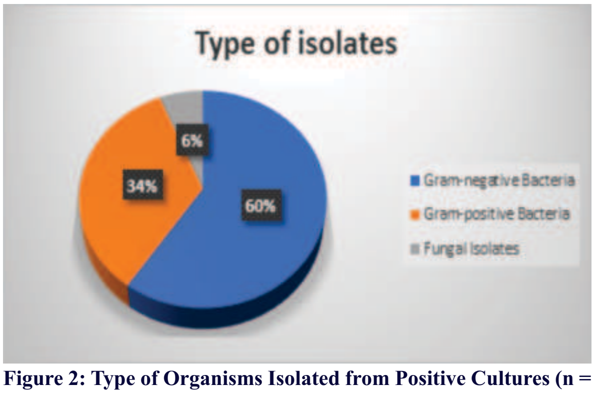

Figure 2: Type of Organisms Isolated from Positive Cultures (n = 415)

Distribution of Isolates

Out of all culture-positive samples bacterial species from eight genera were identified, with Escherichia coli being the most prevalent (36.4%), followed by Staphylococcus aureus (17.3%), Enterococcus (16.1%), and Klebsiella (10.1%). Candida species was isolated in 6.3%(n=26). (Table 1)

Table 1: Distribution of Bacterial Isolates

Genus	Number of Isolates	Percentage (%)
Escherichia coli	151	36.4%
Staphylococcus aureus	72	17.3%
Enterococcus spp.	67	16.1%
Klebsiella spp.	42	10.1%
Non-Fermenters (e.g., Acinetobacter)	22	5.6%
Pseudomonas aeruginosa	14	3.6%
Citrobacter spp.	11	2.8%
Coagulase-negative Staphylococci (CONS)	10	2.6%
Candida spp.	26	6.3%

Antibiotic sensitivity pattern of E.coli isolates: The antibiotic sensitivity pattern of E. coli reveals that Fosfomycin is effective in 96% cases. In contrast, Levofloxacin was mostly ineffective and showing resistance in 75% cases. (Table 2a)

Table 2a: Antibiotic Sensitivity Pattern of E.coli Isolates

Organism	Antibiotic tested	Number of isolates tested for particular antibiotic	Sensitive %	Resistant %
E.coli	Cefazoline	146	28.5 %	61.5 %
	Ciprofloxacin	3	33 %	67 %
	Clotrimazole	151	49 %	51 %
	Fosfomycin	151	96 %	4 %
	Nitrofurantoin	150	96.7 %	3.3 %
	Tetracycline	2	50 %	50 %
	Levofloxacin	4	25 %	75 %

Antibiotic sensitivity pattern of Staphylococcus aureus isolates :The organism showed high sensitivity to Linezolid (100%), Nitrofurantoin (98.5%), and Doxycycline (83.3%). In contrast, it exhibited high resistance to Penicillin (91%). (Table 2b)

Table 2b: Antibiotic Sensitivity Pattern of Staphylococcus Aureus Isolates:

Organism	Antibiotic tested	Number of isolates tested for particular antibiotic	Sensitive %	Resistant %
Staphy-lococcus aureus	Penicillin	72	8 %	91 %
	Doxycycline	72	83.3 %	16.7%
	Linezolid	72	100 %	-
	Cefazoline	4	75 %	25 %
	Ciprofloxacin	02	50 %	50%
	Clotrimazole	71	61 %	39 %
	Fosfomycin	02	100 %	-
	Nitrofurantoin	70	98.5 %	1.5 %

Antibiotic sensitivity pattern of Enterococcus sp. isolates : Enterococcus species showed high sensitivity to Nitrofurantoin (90.6%) and Fosfomycin (84%) but notable resistance to several antibiotics, including Tetracycline, Clotrimazole, Ciprofloxacin, and Levofloxacin. (Table 2c)

Table 2c: Antibiotic Sensitivity Pattern of Enterococcus sp. Isolates:

Organism	Antibiotic tested	Number of isolates tested for particular antibiotic	Sensitive %	Resistant %
Enter-ococcus	Ciprofloxacin	67	36 %	64 %
	Clotrimazole	05	20 %	80 %
	Fosfomycin	67	84 %	16 %
	Nitrofurantoin	64	90.6 %	9.4 %
	Tetracycline	67	30 %	70 %
	Levofloxacin	67	39 %	61%

Antibiotic sensitivity pattern of Klebsiella sp. Isolates: Fosfomycin was most effective against Klebsiella spp. (75% sensitivity), while high resistance was seen to Cefazoline (75%) and Nitrofurantoin (57.6%). (Table 2d)

Table 2d: Antibiotic Sensitivity Pattern of Klebsiella sp. Isolates

Organism	Antibiotic tested	Number of isolates tested for particular antibiotic	Sensitive %	Resistant %

Klebsiella sp.	Cefazoline	40	25 %	75 %
	Clotrimazole	40	50 %	50 %
	Fosfomycin	40	75 %	25 %
	Nitrofurantoin	33	42.4 %	57.6 %
	Tetracycline	2	50 %	50 %

Antibiotic sensitivity pattern of Pseudomonas sp. Isolates : Fosfomycin exhibited the highest sensitivity (87%), while Nitrofurantoin, Tetracycline (87.5%), and Clotrimazole (74%) showed high resistance, with other antibiotics showing low to moderate sensitivity. (Table 2e)

Table 2e: Antibiotic Sensitivity Pattern of Pseudomonas sp. Isolates

Organism	Antibiotic tested	Number of isolates tested for particular antibiotic	Sensitive %	Resistant %
Pseudo-monas sp.	Cefazoline	03	33 %	67 %
	Ciprofloxacin	05	40 %	60 %
	Clotrimazole	19	26 %	74 %
	Fosfomycin	08	87 %	13 %
	Nitrofurantoin	08	12.5 %	87.5 %
	Tetracycline	08	12.5 %	87.5 %
	Levofloxacin	03	33.3 %	66.7 %

Antibiotic sensitivity pattern of non-fermentor group of organisms: Non-fermenting organisms showed moderate sensitivity to Fosfomycin (57%) and Nitrofurantoin (45.9%), with high resistance to Ciprofloxacin, Tetracycline (100%), Cefazoline (65%), and Clotrimazole (60%).(Table 2f)

Table 2f: Antibiotic Sensitivity Pattern of Non-fermentor Group of Organisms

Organism	Antibiotic tested	Number of isolates tested for particular antibiotic	Sensitive %	Resistant %
Non-fermentor group of organisms	Cefazoline	23	35 %	65 %
	Ciprofloxacin	01	-	100 %
	Clotrimazole	25	40 %	60 %
	Fosfomycin	21	57 %	43 %
	Nitrofurantoin	24	45.9 %	54.1 %
	Tetracycline	02	-	100 %
	Levofloxacin	02	50 %	50 %

Antibiotic sensitivity pattern of Citrobacter sp : Citrobacter species showed highest sensitivity to Fosfomycin (87%), followed by Clotrimazole (75%) and Nitrofurantoin (62.5%), while Cefazoline exhibited poor efficacy with 75% resistance.(Table 2g)

Table 2g: Antibiotic Sensitivity Pattern of Citrobacter sp. Isolates:

Organism	Antibiotic tested	Number of isolates tested for particular antibiotic	Sensitive %	Resistant %
Citrobacter sp.	Cefazoline	08	25 %	75 %
	Clotrimazole	08	75 %	25 %
	Fosfomycin	08	87 %	13 %
	Nitrofurantoin	08	62.5 %	37.5 %
	Tetracycline	Nt	-	-
	Levofloxacin	Nt	-	-
nt not tested				

Antibiotic sensitivity pattern of Coagulase Negative Staphylococcus species: High sensitivity was observed in Coagulase Negative Staphylococcus to Linezolid, Clotrimazole, and Nitrofurantoin (100%), followed by Doxycycline (75%). Penicillin showed high resistance (90%). (Table 2h)

Table 2h: Antibiotic Sensitivity Pattern of Coagulase Negative Staphylococcus Species.

Organism	Antibiotic tested	Number of isolates tested for particular antibiotic	Sensitive %	Resistant %
Coagulase Negative Staphy-lococcus	Penicillin	10	10 %	90 %
	Doxycycline	8	75 %	25%
	Linezolid	10	100 %	-
	Cefazoline	Nt	-	-
	Ciprofloxacin	Nt	-	-
	Clotrimazole	03	100 %	-
	Fosfomycin	Nt	-	-
	Nitrofurantoin	3	100 %	-

DISCUSSION

In this study out of total of 1618 urine samples analyzed, 25.6% were culture positive, the remaining 74.4% did not show significant bacterial growth. Growth of candida species was detected in 6.3% samples. These findings are consistent with studies where culture positivity generally ranged between 20% to 40%, depending on patient demographics, specimen collection quality, and prior antibiotic use. A study done by Yigit et al (2014) showed similar positive rate of 28.1%, a key observation in the present study is the female predominance amounting to 68.4% of the positive samples from females compared to 31.6% from males.[8] This gender distribution aligns with well-established epidemiological trends due to anatomical and physiological factors such as a shorter urethra and its proximity to the anus in females. These results closely mirror findings reported by Perween et al.(2022) , who observed a culture positivity rate of 23.5% in urine samples, with a female-to-male ratio of approximately 2.3:1 among the positive cases.[9]

The present analysis revealed that the majority of isolates from urine cultures were Gram-negative bacteria (59.5%), followed by Gram-positive bacteria (34.2%), and fungal isolates (6.3%). In our study Escherichia coli (36.4%) as the primary causative agent of urinary tract infections. These findings align with well-established patterns in UTIs, where Gram-negative organisms, particularly members of the Enterobacteriaceae family, are the predominant uropathogens. The high prevalence of Gram-negative bacteria is consistent with studies such as Perween et al. (2022), who also reported Escherichia coli as the most frequently isolated organism.[9] The dominance of Gram-negative pathogens can be attributed to their unique structural features, such as lipopolysaccharide layers, pili fimbriae and other virulence factors, which facilitate adherence to uroepithelial cells and contribute to virulence.[2] Gram-positive bacteria, accounting for over one-third of the isolates in this study, also play a significant role in UTIs. Among Gram positive Staphylococcus aureus (17.3%) and Enterococcus spp. (16.1%) indicated significant role of Gram-positive cocci in UTIs, particularly in hospitalized patients or those with indwelling catheters. This pattern reflects findings from tertiary care settings, where Gram-positive organisms are increasingly encountered due to shifts in patient profiles and healthcare-associated exposures.[10]

The antibiotic susceptibility patterns observed in this study underscore the evolving landscape of antimicrobial resistance among uropathogens, highlighting both challenges and potential avenues for empirical therapy. E. coli remains the predominant uropathogen, exhibiting high sensitivity to nitrofurantoin (96.7%) and fosfomycin (96%), suggesting their continued efficacy as first-line treatments for uncomplicated UTIs. However, the organism demonstrated significant resistance to cefazoline (61.5%) and clotrimazole (51%). These findings align with a study, which reported high resistance of E. coli to ampicillin (86%) and fluoroquinolones, while noting lower resistance to nitrofurantoin (15.2%) and fosfomycin (27.2%).[11] S. aureus isolates showed complete sensitivity to linezolid (100%) and high sensitivity to nitrofurantoin (98.5%) and doxycycline (83.3%). Conversely, a high resistance to penicillin (91%) was observed. These patterns are consistent with findings from a tertiary care hospital in India, where S. aureus exhibited high resistance to penicillin and variable sensitivity to other antibiotics.[12] Enterococcus isolates showed high sensitivity to Nitrofurantoin (90.6%) and Fosfomycin (84%), indicating their continued efficacy against this organism. However, significant resistance was observed against Tetracycline (70%), Ciprofloxacin (64%), and Clotrimazole (80%). Recent studies have highlighted the efficacy of certain antibiotics against Enterococcus strains isolated from urine samples where nitrofurantoin demonstrated high sensitivity rates, with studies reporting susceptibility in up to 98.1%. Fosfomycin also showed strong activity, with susceptibility rates reaching 94.4% in E. faecalis isolates.[13] Among Klebsiella isolates, fosfomycin showed the highest sensitivity. Clotrimazole and Tetracycline showed moderate sensitivity (50%), although the Tetracycline data were based on only two isolates. High resistance was seen against Cefazoline (75%) and Nitrofurantoin (57.6%), suggesting limited utility of these antibiotics in empirical therapy. Several studies support these findings, with a Pakistani study reporting 77.5% sensitivity of Klebsiella to fosfomycin and only 15.5% to nitrofurantoin, while Serbian data showed 69.57% resistance to cefazoline highlighting regional variability and the limited efficacy of some antibiotics.[14] The Pseudomonas isolates demonstrated high sensitivity to Fosfomycin

(87%), supporting its potential use in treating urinary tract infections (UTIs) caused by Pseudomonas species. However, there was significant resistance to Nitrofurantoin (87.5%) and Tetracycline (87.5%), indicating these antibiotics to be generally ineffective for Pseudomonas UTIs. Moderate sensitivity was observed with fluoroquinolones such as Ciprofloxacin (40%) and Levofloxacin (33.3%), and with Cefazoline (33%), while Clotrimazole showed low efficacy (26%). These results are consistent with previous study reports showing high resistance of Pseudomonas aeruginosa urine isolates to Nitrofurantoin and fluoroquinolone.[15]

These findings underscore the dynamic nature of antimicrobial resistance among uropathogens and highlight the necessity for continuous monitoring of local susceptibility patterns. The variability in resistance profiles across different organisms and regions stresses the importance of evidence-based empirical therapy tailored to the prevailing resistance trends. Additionally, the data emphasize the critical role of infection control practices and antibiotic stewardship programs in curbing the spread of resistant strains in both community and hospital settings.

CONCLUSION

The evolving resistance patterns among uropathogens present a significant challenge to effective clinical management of urinary tract infections. This underscores the urgent need for vigilant antimicrobial stewardship and tailored therapeutic approaches informed by ongoing surveillance. Strengthening these efforts is essential to preserve the efficacy of existing antibiotics and improve patient outcomes in the face of rising antimicrobial resistance.

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