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Original Research Article

Isolation And Antibiotic Sensitivity Pattern to Urinary Pathogens in A Tertiary Care Hospital in South India

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ABSTRACT

Background: Urinary tract infections (UTIs) are among the most common bacterial infections and are increasingly complicated by antimicrobial resistance. **Objective:** To determine the prevalence, etiological profile, and antimicrobial susceptibility patterns of urinary isolates in a tertiary care hospital. **Methods:** A prospective study was conducted on urine samples processed for culture and antimicrobial susceptibility testing from January to December 2024. Isolates were identified using standard microbiological methods, and susceptibility testing was performed according to CLSI guidelines. **Results:** Of 3274 urine samples processed, 766 yielded significant growth (23.4%). A total of 770 isolates were recovered. Females accounted for 53.9% of culture-positive cases. Gram-negative bacilli predominated (79.1%), followed by Gram-positive cocci (15.1%) and *Candida* species (5.8%). *Escherichia coli* was the most common isolate (50.5%), followed by *Klebsiella* spp. (11.9%) and *Enterococcus* spp. (10.6%). Enterobacterales demonstrated high susceptibility to Fosfomycin (87.9%), Piperacillin–tazobactam (87.8%), Tobramycin (87.3%), Amikacin (81.2%), Nitrofurantoin (80.3%) and Imipenem (79.2%), whereas high resistance was observed to ampicillin, cephalosporins, cotrimoxazole and fluoroquinolones. *Enterococcus* species demonstrated high susceptibility to Vancomycin (86.6%) and Linezolid (93.9%). **Conclusion:** *Escherichia coli* remained the predominant uropathogen. Continuous surveillance of local antimicrobial susceptibility patterns is essential to guide empirical therapy and antimicrobial stewardship.

Keywords*: Urinary tract infection; Uropathogens; Antimicrobial susceptibility; Antimicrobial stewardship.*

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in clinical practice. The spectrum of uropathogens and their antimicrobial susceptibility patterns vary across geographical regions and healthcare settings. In many healthcare settings, empirical antibiotic therapy is frequently initiated before culture results become available, particularly in patients requiring urgent treatment. However, inappropriate empirical antimicrobial use can contribute to the emergence and spread of multidrug-resistant organisms. Therefore, regular surveillance of local uropathogen distribution and antimicrobial susceptibility patterns is essential for guiding empirical therapy and promoting rational antibiotic use.[1] The present study aimed to determine the bacterial profile of urinary tract infections and evaluate the antimicrobial susceptibility patterns of the isolated pathogens in a tertiary care hospital.

Objectives: 1. To identify the various urinary pathogens isolated from urine samples and to determine the antimicrobial susceptibility of isolated uropathogens.

MATERIALS AND METHODS

Study design and setting: A prospective study was conducted in the Department of Microbiology, Arunai Medical College and Hospital, Tiruvannamalai, Tamil Nadu, India, over a period of one year from January 2024 to December 2024.

Study population and sample size: Patients attending outpatient departments and admitted in wards with suspected urinary tract infection (UTI) whose urine samples were received for culture and sensitivity testing during the study period were included. Convenience sampling was used and all consecutive eligible urine samples received in the microbiology laboratory were analyzed.

Inclusion and exclusion criteria: Patient aged >18 years, urine samples received for culture and antimicrobial susceptibility testing during the study period were included and patients aged <18years, contaminated, duplicate, improperly collected, samples with incomplete data were excluded in this study.

Sample collection and processing: Clean catch, mid-stream urine samples were collected in sterile wide-mouthed containers and transported to the microbiology laboratory. Samples were processed within 2 hours of collection; specimens with delayed processing were refrigerated at 2-8°C. Urine samples were cultured using the semi-quantitative calibrated loop technique (0.001mL) on Cystine Lactose Electrolyte Deficient (CLED) Agar and 5% Sheep Blood Agar plates and the plates were incubated aerobically at 37°C for 18-24 hours.[2] Significant bacteriuria was defined as the growth of a single bacterial species at a concentration of $\geq 10^5$ colony-forming units (CFU)/mL of urine. Colony counts between 10^4 and 10^5 CFU/mL were interpreted in conjunction with clinical findings, whereas counts $<10^4$ CFU/mL were considered insignificant. For catheterized urine samples, a bacterial count of $\geq 10^3$ CFU/mL in symptomatic patients was considered significant.[3] Bacterial isolates were identified based on colony morphology, Gram staining, and standard biochemical tests.

Antimicrobial Susceptibility tests: Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100, 34th edition(2024) guidelines and reported as Susceptible (S), Intermediate (I), or Resistant (R). Antimicrobial agents tested for each organism were selected based on CLSI organism-specific recommendations (Tier 1–Tier 4 reporting groups) for Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter* spp, *Staphylococcus* spp, *Enterococcus* spp, Beta hemolytic *Streptococcus* spp and susceptibility results were analyzed following the CLSI-recommended cascade reporting approach to support antimicrobial stewardship. Intrinsic resistance patterns were considered during interpretation and analysis of antimicrobial susceptibility data.[2] Antimicrobial agents tested for each organism group are shown in Table 1.

Table 1. Antimicrobial Agents Tested According to CLSI 2024 Recommendations and Study Protocol

Organism Group	Tier 1	Tier 2	Tier 3	Tier 4
Enterobacterales	Ampicillin, Amoxicillin-clavulanate, Cefazolin, Ceftriaxone, Piperacillin-tazobactam, Gentamicin, Ciprofloxacin, Cotrimoxazole, Nitrofurantoin	Cefuroxime, Cefepime, Amikacin, Tobramycin, Imipenem, Meropenem, Tetracycline	Fosfomycin*	Ceftazidime Aztreonam
Pseudomonas aeruginosa	Ceftazidime, Cefepime, Piperacillin-tazobactam, Tobramycin, Ciprofloxacin	Imipenem, Amikacin	Ceftazidime-avibactam	Aztreonam
Acinetobacter spp.	Ampicillin-sulbactam, Ceftazidime, Cefepime, Ciprofloxacin, Gentamicin, Tobramycin, Tetracycline	Imipenem, Amikacin, Piperacillin-tazobactam, Trimethoprim-sulfamethoxazole	-	Doxycycline, Ceftriaxone, Colistin
Staphylococcus spp.	Clindamycin, Oxacillin, Doxycycline, Tetracycline, Erythromycin, Cotrimoxazole,	Penicillin, Linezolid	-	Ciprofloxacin

	Nitrofurantoin, Vancomycin			
Enterococcus spp.	Ampicillin, Penicillin, Nitrofurantoin	High-level Gentamicin(High-level), Vancomycin, Linezolid, Ciprofloxacin	Fosfomycin, Tetracycline	-
β-Hemolytic *Streptococcus* spp.	Clindamycin, Erythromycin, Penicillin	Tetracycline	Vancomycin, Ceftriaxone	Linezolid, Cefepime, Levofloxacin

*** Fosfomycin tested only for E. coli isolates in Enterobacterales and *Enterococcus* species**
(* Antimicrobial agents were selected and interpreted according to CLSI M100, 34th edition (2024))

Statistical analysis: Data were entered into Microsoft Excel 2019 and analyzed using descriptive statistics. Results were presented as frequencies and percentages.

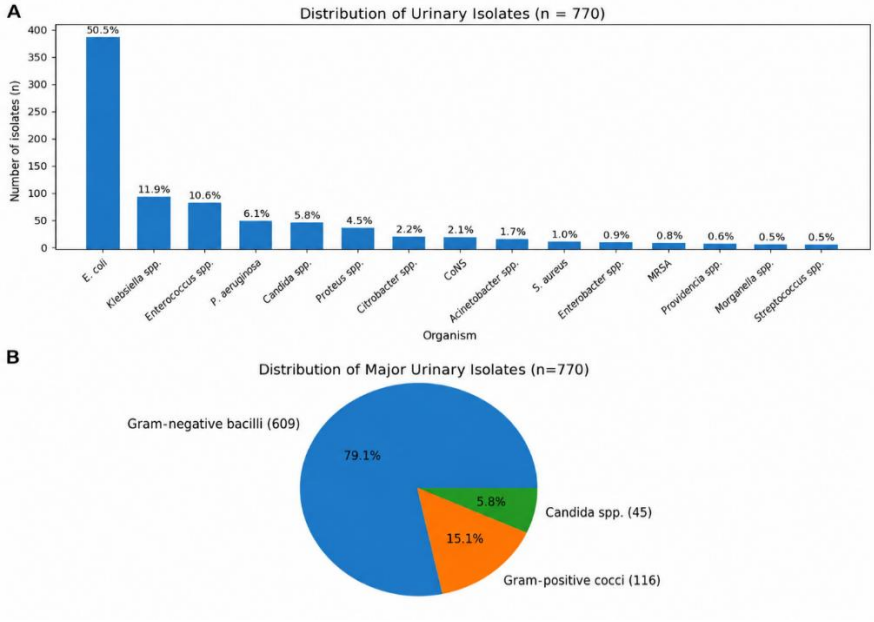
Ethical considerations: The study was reviewed and approved by the Institutional Ethics Committee of Arunai Medical College and Hospital, Tiruvannamalai.

RESULTS

Culture Positivity and Isolate Distribution

During the study period from January 2024 to December 2024, a total of 3274 urine samples were processed for culture and antimicrobial susceptibility testing. Among these, 766 samples yielded significant microbial growth, resulting in a culture positivity rate of 23.4%. A total of 770 microbial isolates were recovered, including four polymicrobial cultures (0.52%). Among culture-positive cases, 413 (53.9%) were females and 353 (46.1%) were males. Gram-negative bacilli (GNB) constituted the majority of isolates (609/770, 79.1%), followed by Gram-positive cocci (GPC) (116/770, 15.1%) and *Candida* species (45/770, 5.8%). *Escherichia coli* was the predominant isolate accounting for 389 (50.5%) isolates, followed by *Klebsiella* spp. 92 (11.9%), *Enterococcus* spp. 82 (10.6%), followed by *Pseudomonas aeruginosa* 47 (6.1%), *Candida* spp. 45 (5.8%), *Proteus* spp. 35 (4.5%), *Citrobacter* spp. 17 (2.2%), coagulase-negative staphylococci (CoNS) 16 (2.1%), *Acinetobacter* spp. 13 (1.7%), *Staphylococcus aureus* 8 (1.0%), *Enterobacter* spp. 7 (0.9%), *Methicillin-resistant Staphylococcus aureus (MRSA)* 6 (0.8%), *Providencia* spp. 5 (0.6%), *Morganella morganii* 4 (0.5%), and *Streptococcus* spp. 4 (0.5%). The distribution of isolates and major microbial groups is represented in Figure 1A and 1B, respectively.

Figure 1 – (A) Distribution of Urinary Isolates (n=770) (B) Distribution of Major Microbial Groups (n=770)



Enterobacterales Isolates and Antimicrobial Susceptibility Pattern

A total of 549 Enterobacterales isolates were recovered, comprising *Escherichia coli* (n=389), *Klebsiella* spp. (n=92), *Proteus* spp. (n=35), *Citrobacter* spp. (n=17), *Enterobacter* spp. (n=7), *Providencia* spp. (n=5), and *Morganella morganii* (n=4). Antimicrobial Susceptibility Pattern of Enterobacterales Isolates shown in Figure 2.

***Escherichia coli* (n=389)**

Escherichia coli was the predominant Enterobacterales isolate, accounting for 70.9%. Highest susceptibility was observed to fosfomycin (87.9%), piperacillin–tazobactam (87.1%), tobramycin (85.9%), amikacin (84.3%), meropenem (84.1%), imipenem (81.2%), and nitrofurantoin (80.5%). High resistance was observed to ampicillin (91.3%), cefazolin (87.7%), cotrimoxazole (83.8%), cefuroxime (83.5%), ciprofloxacin (76.1%), and ceftriaxone (75.8%).

***Klebsiella* spp. (n=92)**

Klebsiella spp. represented 16.8% of Enterobacterales isolates and comprised *Klebsiella pneumoniae* (n=81) and *Klebsiella oxytoca* (n=11). Highest susceptibility was demonstrated to tobramycin (93.5%), piperacillin–tazobactam (85.9%), meropenem (80.4%), imipenem (78.3%), and amikacin (70.7%). Resistance was highest to cefazolin (89.1%), cefuroxime (82.6%), ciprofloxacin (76.1%), cotrimoxazole (75.0%), ceftriaxone (70.7%), and ceftazidime (69.6%). Ampicillin susceptibility was not interpreted, as *Klebsiella* species are intrinsically resistant to ampicillin.

***Proteus* spp. (n=35)**

Proteus spp. accounted for 6.4% of Enterobacterales isolates and comprised *Proteus mirabilis* (n=29) and *Proteus vulgaris* (n=6). Piperacillin–tazobactam (88.6%), tobramycin (85.7%), cefepime (82.9%), and amikacin (77.1%) showed the highest activity. Resistance was highest to cefazolin (85.7%), cotrimoxazole (77.1%), ampicillin/amoxicillin (65.7%), and cefuroxime (60.0%).

Intrinsic resistance patterns were considered during interpretation. *P. mirabilis* is intrinsically resistant to nitrofurantoin, tetracycline, tigecycline, polymyxin B, and colistin. *P. vulgaris* is intrinsically resistant to ampicillin, first- and second-generation cephalosporins, nitrofurantoin, tetracycline, tigecycline, polymyxin B, and colistin; therefore, susceptibility results for these agents were not interpreted.

***Citrobacter* spp. (n=17)**

Citrobacter spp. comprised *Citrobacter koseri* (n = 14) and *Citrobacter freundii* (n = 3). *Citrobacter* isolates demonstrated excellent susceptibility to piperacillin–tazobactam (100%), imipenem (94.1%), meropenem (88.2%), amoxicillin–clavulanate (82.4%), cefepime (82.4%), amikacin (82.4%), and cotrimoxazole (82.4%). Resistance was highest to cefazolin (82.4%) and nitrofurantoin (47.1%).

Intrinsic resistance patterns were considered during interpretation. *Citrobacter koseri* is intrinsically resistant to ampicillin and ticarcillin. *Citrobacter freundii* is intrinsically resistant to ampicillin, amoxicillin–clavulanate, ampicillin–sulbactam, first- and second-generation cephalosporins, and cephamycins; therefore, susceptibility results for these agents were not interpreted.

***Enterobacter* spp. (n=7)**

Enterobacter spp. comprised *Klebsiella aerogenes* (formerly *Enterobacter aerogenes*) (n = 5) and *Enterobacter cloacae* complex (n = 2). *Enterobacter* isolates exhibited complete resistance to cefuroxime and nitrofurantoin (100% each). Piperacillin–tazobactam demonstrated complete susceptibility (100%), followed by amikacin (85.7%), cefepime (71.4%), and ciprofloxacin (71.4%). Ceftriaxone resistance was high (85.7%).

Enterobacter cloacae complex and K. aerogenes are intrinsically resistant to ampicillin, amoxicillin–clavulanate, ampicillin–sulbactam, first-generation cephalosporins, and cephamycins; therefore, susceptibility results for these agents were not interpreted.

***Morganella morganii* (n=4)**

Morganella morganii showed complete susceptibility to amikacin and piperacillin–tazobactam (100% each). Moderate susceptibility was observed to ceftriaxone, cefepime, gentamicin, and ciprofloxacin (75% each). Reduced susceptibility was noted for carbapenems and cotrimoxazole (50% each).

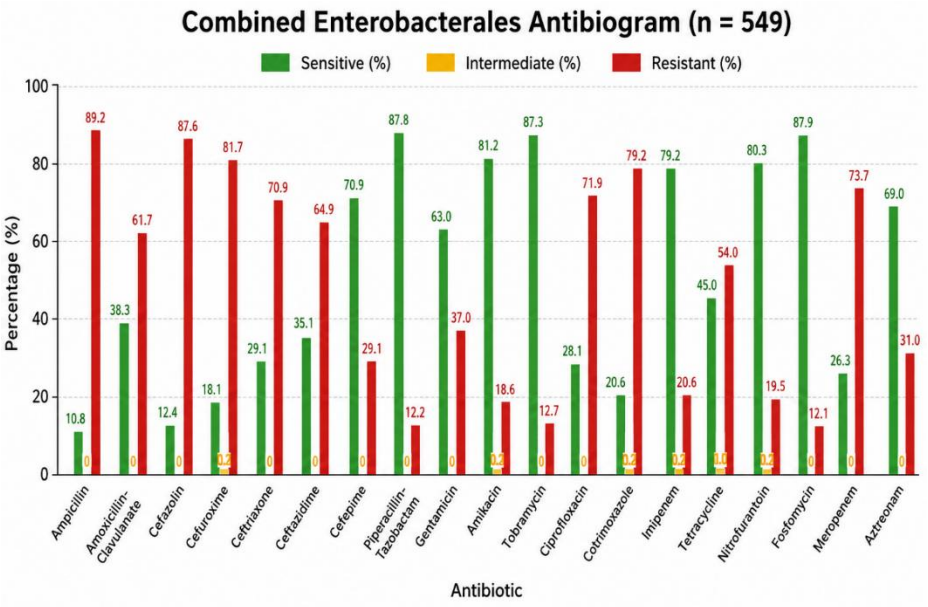
Intrinsically resistant to ampicillin, amoxicillin–clavulanic acid, first- and second-generation cephalosporins, nitrofurantoin, tigecycline, polymyxin B, and colistin; therefore, susceptibility results for these agents were not interpreted.

***Providencia* spp. (n=5)**

Providencia rettgeri demonstrated complete susceptibility to piperacillin–tazobactam and tobramycin (100% each). Moderate susceptibility was observed to carbapenems, amikacin, cotrimoxazole (60% each). High resistance was observed to cefuroxime, ceftriaxone, cefepime, ciprofloxacin, and gentamicin.

Intrinsically resistant to ampicillin, amoxicillin–clavulanate, first-generation cephalosporins, tetracycline, tigecycline, nitrofurantoin, polymyxin B, and colistin; therefore, susceptibility results for these agents were not interpreted.

Figure 2: Antimicrobial Susceptibility and Resistance patterns among Enterobacterales Isolates (n=549)

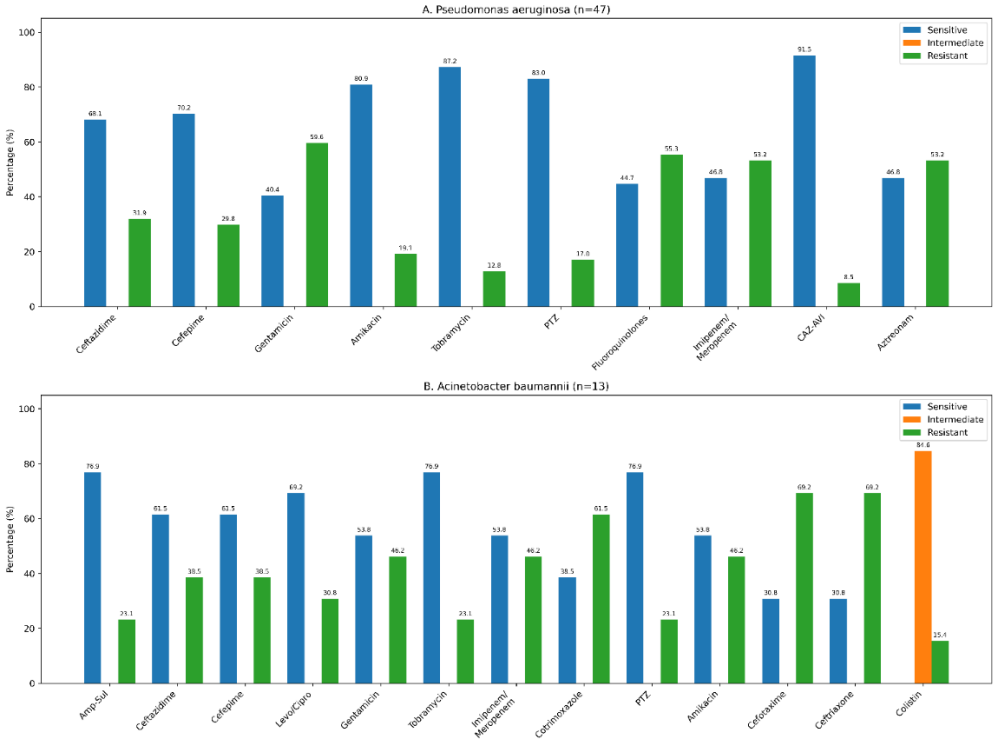


Non-Fermenting Gram-Negative Bacilli

Pseudomonas aeruginosa accounted for 47 (6.1%) isolates. Highest susceptibility was observed to ceftazidime–avibactam (91.5%), tobramycin (87.2%), piperacillin–tazobactam (83.0%), and amikacin (80.9%). Resistance was highest to gentamicin (59.6%), fluoroquinolones (55.3%), carbapenems (53.2%), and aztreonam (53.2%).

Acinetobacter baumannii accounted for 13 (1.7%) isolates. Highest susceptibility was observed to ampicillin–sulbactam (76.9%), piperacillin–tazobactam (76.9%), and tobramycin (76.9%). High resistance was observed to cefotaxime (69.2%), ceftriaxone (69.2%), cotrimoxazole (61.5%), and carbapenems (46.2%). Colistin demonstrated predominantly intermediate susceptibility (84.6%). Antimicrobial Susceptibility interpretation of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* shown in Figure 3(A & B) respectively.

Figure 3- (A & B)



Gram-Positive Cocci and Antimicrobial Susceptibility Pattern

A total of 116 Gram-positive cocci were isolated. *Enterococcus* spp. was the predominant Gram-positive organism accounting for 82 (70.7%) isolates, followed by Coagulase-negative staphylococci (CoNS) 16 (13.8%), *Staphylococcus aureus* 8 (6.9%), MRSA 6 (5.2%), and *Streptococcus* spp. 4 (3.4%). The antimicrobial susceptibility patterns of Gram-positive cocci are presented in Table 2.

Table 2 Combined Gram-Positive Antibiogram (S/I/R %)

Antibiotic	*Enterococcus*	*S. aureus*	*MRSA*	CoNS	*Streptococcus*
Ampicillin/Penicillin	58.5/0/41.5	12.5/0/87.5	0/0/100	0/0/100	50/25/25
Oxacillin	NA	100/0/0	0/0/100	0/0/100	NA
Clindamycin	NA	87.5/0/12.5	66.7/0/33.3	43.8/0/56.2	25/25/50
Erythromycin	NA	25/25/50	0/16.7/83.3	0/0/100	0/0/100
Doxycycline	NA	75/0/25	33.3/0/66.7	56.2/0/43.8	NA
Tetracycline	35.4/0/64.6	50/0/50	33.3/0/66.7	18.8/0/81.2	0/25/75
Co-trimoxazole	NA	75/0/25	66.7/0/33.3	43.8/0/56.2	NA
Nitrofurantoin	37.8/0/62.2	0/0/100	33.3/0/66.7	31.2/0/68.8	NA
HLG	69.5/0/30.5	NA	NA	NA	NA
Ciprofloxacin/ Levofloxacin	29.3/0/70.7	27.3/1.7/71.0	50/0/50	56.3/0/43.7	50/25/25
Vancomycin	86.6/0/13.4	100/0/0	100/0/0	87.5/0/12.5	100/0/0
Linezolid	93.9/0/6.1	100/0/0	100/0/0	100/0/0	100/0/0
Ceftriaxone	NA	NA	NA	NA	50/0/50
Cefepime	NA	NA	NA	NA	100/0/0
Fosfomycin	31.7/0/68.3	NA	NA	NA	NA

- **Fosfomycin susceptibility testing was performed only for *Enterococcus* spp. among Gram-positive cocci.**

***Enterococcus* spp. (n = 82)**

Enterococcus spp. showed highest susceptibility to linezolid (93.9%) and vancomycin (86.6%). High resistance was observed to ciprofloxacin/levofloxacin (70.7%), tetracycline (64.6%), and nitrofurantoin (62.2%). Intrinsic resistance (IR) to cephalosporins, clindamycin, cotrimoxazole, fusidic acid, and gentamicin was observed. Fosfomycin susceptibility testing was performed only for Enterococcus spp., with 31.7% susceptibility and 68.3% resistance.

***Staphylococcus aureus* (n = 8)**

S. aureus isolates were fully susceptible to oxacillin, vancomycin, and linezolid (100% each). High susceptibility was also observed to clindamycin (87.5%), doxycycline (75%), and cotrimoxazole (75%).

***MRSA* (n = 6)**

All MRSA isolates were resistant to penicillin and oxacillin (100%) but remained fully susceptible to vancomycin and linezolid (100%). Susceptibility to clindamycin and cotrimoxazole was 66.7%, while 50% of isolates were susceptible to ciprofloxacin/levofloxacin.

CoNS (n = 16)

CoNS demonstrated complete susceptibility to linezolid (100%) and high susceptibility to vancomycin (87.5%). High resistance was observed to oxacillin (100%), erythromycin (100%), tetracycline (81.2%), and nitrofurantoin (68.8%).

***Streptococcus* spp. (n = 4)**

Streptococcus spp. were fully susceptible to vancomycin, linezolid, and cefepime (100%). Resistance was highest to erythromycin (100%) and tetracycline (75%), while 50% resistance was observed to clindamycin and ceftriaxone.

***Candida* Isolates**

Candida species accounted for 45 (5.8%) isolates recovered during the study period and represented the most common fungal pathogen isolated from urine cultures.

DISCUSSION

The culture positivity rate in the present study was 23.4%, which is comparable to rates reported by Ahirwar et al. (22.93%).[8] Some studies reported lower positivity rates of 16.1% and 19.83% respectively[4,5] and some studies reported higher prevalence rate of 28% and 41.8%.[2,6] Differences in positivity rates may be attributed to variations in patient populations, study settings, and diagnostic practices.[6] Females constituted 53.9% of culture-positive cases compared with males 46.1%, similar to previous studies that reported higher positivity rates among females, reflecting the higher susceptibility of women to urinary tract infections due to anatomical and physiological factors.[1,2]

Gram-negative bacilli were the predominant isolates (79.1%), followed by Gram-positive cocci (15.1%) and Candida species (5.8%). This distribution is consistent with previous studies reporting Gram-negative organisms accounted for the majority of uropathogens.[5,8] Among Gram- negative isolates, *Escherichia coli* was the most prevalent pathogen, followed by *Klebsiella species* and *Pseudomonas aeruginosa*, whereas *Enterococcus* spp was predominant among Gram-positive cocci. Similar organism distributions have been reported in earlier studies.[1,4] The predominance of *E. coli* may be attributed to its virulence factors, including adhesins and biofilm-forming ability, which facilitate colonization of the urinary tract.

Among Enterobacterales, high susceptibility was observed to fosfomycin, nitrofurantoin, aminoglycosides, piperacillin–tazobactam, and carbapenems, whereas resistance was high to ampicillin, cephalosporins, cotrimoxazole, and fluoroquinolones. Similar resistance trends have been reported in recent studies.[1,7]

Non-fermenting Gram-negative bacilli accounted for 7.8% of isolates. *Pseudomonas aeruginosa* demonstrated good susceptibility to ceftazidime–avibactam, piperacillin–tazobactam, and aminoglycosides. Comparable findings were reported by Berwal et al., highlighting the continued effectiveness of these agents against non-fermenters.[9]

Gram-positive cocci represented 15.1% of isolates, with *Enterococcus* spp. accounting for 70.7% of Gram-positive isolates. Similar predominance of *Enterococcus* among Gram-positive uropathogens has been documented by various studies.[1,5] Enterococcal isolates in the present study retained good susceptibility to vancomycin, and linezolid.

Candida species accounted for 5.8% of isolates, which is comparable to the 5–10% prevalence reported in tertiary-care studies. The occurrence of candiduria may be associated with hospitalization, diabetes mellitus, prolonged antimicrobial therapy, and urinary catheterization.[7]

Based on the observed susceptibility pattern, fosfomycin and nitrofurantoin appear suitable options for empirical treatment of uncomplicated urinary tract infections, while piperacillin–tazobactam, aminoglycosides, and carbapenems may be reserved for complicated infections. Continuous surveillance of local antimicrobial susceptibility patterns is essential to guide empirical therapy and antimicrobial stewardship practices.

Limitations

This study was conducted at a single tertiary care center and may not fully represent regional antimicrobial resistance patterns. Molecular characterization of antimicrobial resistance mechanisms, including ESBL, AmpC beta-lactamase and carbapenemase production was not performed.

CONCLUSION

The present study highlights the predominance of Gram-negative bacilli, particularly *Escherichia coli*, as the principal uropathogens in urinary tract infections. Enterobacterales demonstrated high susceptibility to fosfomycin, nitrofurantoin, aminoglycosides, piperacillin–tazobactam, and carbapenems, while considerable resistance was observed to ampicillin, cephalosporins, cotrimoxazole, and fluoroquinolones. *Enterococcus* spp. emerged as the leading Gram-positive pathogen and retained excellent susceptibility to vancomycin and linezolid. These findings emphasize the importance of continuous surveillance of local antimicrobial susceptibility patterns and the use of culture-guided therapy to optimize patient management and support antimicrobial stewardship initiatives.

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