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Original Article

Microbiology Section

Evolving Landscape in Urinary Pathogens and Antimicrobial Susceptibility over Five Years: A Retrospective Observational Study

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ABSTRACT

Introduction: Urinary Tract Infections (UTIs) are among the most common bacterial infections in both community and hospital settings. The rising burden of antimicrobial resistance among uropathogens poses a challenge to empirical therapy, particularly in resource-limited settings. Continuous regional surveillance is essential to guide effective treatment strategies.

Aim: To evaluate the changing spectrum of urinary pathogens and their antimicrobial susceptibility patterns through analysis of urine culture isolates.

Materials and Methods: This retrospective observational study was conducted in the Department of Microbiology, Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospital, Pune, Maharashtra, India from January 2020 to December 2024 on urine culture samples. All outpatients and inpatients presenting to the hospital during the study period with suspected UTI, based on urine culture findings, were included. Semiquantitative culture on Cystine-Lactose-Electrolyte-Deficient (CLED) agar was used to isolate significant bacteriuria ($\geq 10^5$ Colony-Forming Units (CFU)/mL), followed by Gram staining, biochemical identification and Kirby-Bauer disc diffusion antibiotic susceptibility testing. Demographic parameters, seasonal patterns, and antimicrobial resistance data were analysed. Negative binomial regression and the Cochran-Armitage trend test were applied as appropriate. A p-value of <0.05 was considered statistically significant.

Results: A total of 26,045 urine samples were processed, of which 4,248 (16.33%) showed substantial bacterial growth. Of culture-positive cases, 2,219 (52.2%) were females. Most positive cultures were from inpatient settings, 3,466 (81.6%), followed by intensive care units, 417 (9.8%), and Outpatient Departments (OPD), 365 (8.6%). The highest incidence of positive cultures was in the 19-30 years age group, at 835 (19.7%). The lowest incidence was observed in the 13-18 years age group, at 116 (2.7%). *E. coli* was the most common uropathogen, 1834 (43.2%), followed by *K. pneumoniae*, 876 (20.6%), and *Enterococcus* spp. 891 (21.0%). High resistance was recorded among *E. coli* isolates to ampicillin 1680 (92.3%), cefotaxime 1656 (91.9%), and ceftriaxone 1606 (89.6%). High resistance was observed among *Enterococcus* spp. to ciprofloxacin 523 (78.2%) and norfloxacin 410 (77.7%). Nitrofurantoin demonstrated lower resistance than other antimicrobials in *E. coli* 288 (23.3%) and *Enterococcus* spp. 163 (23.2%), suggesting retained efficacy for some UTIs.

Conclusion: The study highlights evolving antimicrobial resistance patterns in uropathogens over five years, with increasing resistance to commonly prescribed empirical antibiotics, particularly cephalosporins and fluoroquinolones. Despite this, nitrofurantoin, linezolid, vancomycin, and selected reserve drugs maintained better susceptibility profiles against several isolates. Routine surveillance, evidence-based empirical therapy, institution-specific antibiograms and antimicrobial stewardship are essential to optimise clinical outcomes and curb the emergence of antimicrobial resistance.

Keywords: Antibiotic susceptibility pattern, Antimicrobial resistance, Urinary tract infections, Uropathogens

INTRODUCTION

The UTIs are among the most common bacterial infections globally, affecting millions of individuals each year across all age groups and genders, though with a significantly higher prevalence in women due to factors such as a shorter urethra, absence of bactericidal prostatic secretions, a higher bacterial load in the urothelial mucosa, and pregnancy [1]. In clinical practice, the severity of UTIs varies widely, ranging from localised cystitis to the more invasive pyelonephritis. If not managed properly, these upper tract infections carry a high-risk of morbidity and can rapidly progress to life-threatening urosepsis. While the aetiological landscape includes fungal and rare viral pathogens, the vast majority, remain bacterial in origin, necessitating a robust antibiotic stewardship approach [2]. UTIs are the leading cause of healthcare-associated infections among hospitalised patients and the second leading cause of hospital visits [3]. UTIs are predominantly caused by a variety of microbial pathogens. *Escherichia coli* is the most common uropathogen in both community and healthcare settings, followed by other Enterobacterales, such as *Klebsiella* spp. and *Pseudomonas* spp. [4]. Gram-positive organisms, such as *Enterococcus* spp., are less common but are frequently seen in healthcare settings and in adults with chronic indwelling catheters [5]. Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli*, a major multidrug-resistant organism, is responsible for severe infections in both hospital and community settings, especially in lower-middle-income countries with poor hygiene and sanitation [6].

Due to the 24-48 hour diagnostic gap between clinical presentation and obtaining definitive culture and sensitivity results, empirical antibiotic treatment remains the standard of care due to diagnostic lag for suspected UTIs [7]. This approach is necessary as the precise causative agent and its susceptibility profile are unknown at the time of initial presentation, which dictates the bulk of antibiotic usage prior to culture confirmation [8]. Effective management of UTIs is increasingly complicated by the emergence of diverse uropathogens and their evolving resistance to antimicrobial agents [9]. This necessitates ongoing monitoring to inform and optimise empirical therapy guidelines according to the region [10].

Overuse and misuse of antibiotics have invariably increased resistance factors, especially in outpatient settings. Consequently, drug resistance has become alarmingly widespread in several regions, with resistance to commonly used antibiotics. Most current issues affecting treatment include the emergence of ESBL- producing strains of *E. coli* and other uropathogens in the community. Such multiresistant bugs are resistant to a wide range of β-lactam antibiotics, including penicillins and cephalosporins, which have long been standard anti-UTI agents [11].

The UTIs continue to be a major cause of global antimicrobial consumption; however, the growing prevalence of resistant uropathogens increasingly undermines the efficacy of empirical treatment protocols. Although surveillance studies frequently document resistance patterns, their practical implications for clinical decision-making and robust antimicrobial stewardship remain underdeveloped. While national surveillance frameworks, such as the ICMR Antimicrobial Resistance Surveillance Network (AMRSN) and the WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS), provide a macro-level perspective on resistance trends in India, these broad datasets can mask significant inter-hospital and intra-regional variations [12,13]. Consequently, local institutional antibiograms remain the superior tool for guiding empiric therapy and establishing effective hospital-specific antimicrobial stewardship programs. UTIs continue to be a major reason for the use of antimicrobials, although the growing resistance of uropathogens makes empirical therapy less effective [10]. Surveillance studies often record resistance patterns; however, their practical ramifications for clinical decision-making and stewardship are frequently inadequately examined.

The study aimed to evaluate the changing spectrum of urinary pathogens and their antimicrobial susceptibility patterns over five years by analysing urine culture isolates.

MATERIALS AND METHODS

A retrospective observational study was conducted in the Department of Microbiology, Byramjee Jeejeebhoy Government Medical College and Sassoon General Hospital, Pune, Maharashtra, India. Urine culture data collected between January 2020 and December 2024 were retrieved from the WHONET database and the Urine Culture and Sensitivity registers. The data were analysed between September 2025 and November 2025. Ethical clearance was obtained prior to the study (IEC approval number: BJGMC/IEC/Pharmac/ND-0925311-311). The dataset was standardised and validated against hospital records, with duplicates and incomplete entries removed. After strict de-identification and deletion of source records to ensure patient privacy, the final analysis was conducted on variables including age, gender, microbial growth, and antimicrobial susceptibility results. As this was a time-bound study, all urine samples from patients with suspected UTIs fulfilling the inclusion criteria between 2020 and 2024 were included in the study.

Inclusion criteria: Patients of all ages and genders who presented with suspected UTIs, characterised by clinical indicators such as dysuria, increased urinary frequency, or fever, were included. This also included pregnant women presenting with suspected UTI during antenatal visits. Only the initial isolate per patient was included. For the purpose of this study, pregnant individuals presenting with suspected UTI were integrated into the overall study group to provide a comprehensive assessment of regional resistance patterns.

Exclusion criteria: Specimens yielding polymicrobial or mixed growth were excluded. Furthermore, the study omitted samples failing to meet the diagnostic threshold for significant bacteriuria (10^5 CFU/mL) [14]. Incomplete clinical or laboratory documentation were omitted to maintain the integrity of the final analytical dataset.

Study Procedure

Specimens were obtained via clean-catch midstream or catheterised collection and kept at 2-8°C until laboratory processing was initiated within a 12-hour window from the time of receipt. Semiquantitative plating was done for all cultures, inoculation of 0.001 mL of urine onto CLED agar via a calibrated loop, followed by overnight incubation at 37°C. Significant bacteriuria was defined as the presence of ≥10^5 CFU per mL of a single bacterial species in a clean-catch midstream urine sample according to standard Kass criteria and processed [14]. Bacterial identification was performed using standard microbiological and biochemical methods. Preliminary identification included Gram stain, catalase test, oxidase test and motility. Biochemical characterisation was performed using Indole, Methyl Red, Voges–Proskauer, citrate utilisation, urease and Triple Sugar Iron (TSI) tests, and isolates were identified to the species level based on standard biochemical characteristics.

Antimicrobial Susceptibility Testing (AST) was conducted via the Kirby-Bauer disk diffusion on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) M100 guidelines 2020-2024. The inoculated plates were incubated aerobically at 35 ± 2°C for 16-18 hours, following which the zone diameters were measured and interpreted as susceptible, intermediate, or resistant according to CLSI criteria [15]. *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used as quality control for routine AST. Colistin susceptibility among Gram-negative isolates was determined by Broth Microdilution (BMD), with resistance defined as a Minimum Inhibitory Concentration (MIC) ≥4 µg/mL per CLSI M100 guidelines [15]. For the identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA), the cefoxitin disk (30 µg) diffusion method was used, and a zone <22 mm according to CLSI breakpoints. *S. aureus* ATCC 43300- as a control for specialised detection of methicillin resistance, served as the control for Cefoxitin Disk Diffusion (CDD). For Vancomycin-Resistant *Enterococcus* (VRE) screening, the Vancomycin agar screen (6 µg/mL) test was initially performed, with all resistant phenotypes subsequently confirmed by the MIC method. *Enterococcus faecalis* ATCC 51299- as a control for vancomycin susceptibility testing and the validation of vancomycin screen agar.

For highly resistant Gram-negative bacilli, colistin susceptibility was assessed via BMD, as disk diffusion is not recommended for accurate colistin profiling due to poor agar diffusion. All susceptibility results and resistance phenotypes were interpreted in strict accordance with the CLSI M100 benchmarks [15]. Phenotypic screening for ESBL production was performed using the Double-Disk Synergy Test (DDST). Ceftazidime (30 µg) and cefotaxime (30 µg), alone and in combination with clavulanate (10 µg), were used. An increase of ≥5 mm in the inhibition zone diameter of ceftazidime or cefotaxime tested in combination with clavulanic acid, compared to the zone diameter of the respective antibiotic tested alone, was interpreted as confirmation of ESBL production by the isolate. *Klebsiella pneumoniae* ATCC 700603- as positive control for DDST [15]. All bacterial isolates were classified as MDR and XDR based on standard definitions [16]. MDR: Non-susceptibility to at least one agent in three or more antimicrobial categories. Extensive Drug Resistance (XDR): Non-susceptibility to at least one agent in all but two or fewer antimicrobial categories.

STATISTICAL ANALYSIS

Data management and analysis of antimicrobial susceptibility were performed using WHONET 2024, Microsoft Excel® 2021 and R statistical software. Descriptive statistics were used for statistical analysis. The negative binomial regression and Cochran-Armitage trend test were applied as appropriate. A p-value of <0.05 was considered statistically significant.

RESULTS

During the five-year study period, 26,045 urine samples were processed. Of these, 4,248 (16.3%) urine cultures showed significant bacterial growth. Among the positive cultures, 2,219 (52.2%) were from women and 2029 (47.8%) from men. Among culture-positive

cases, 3466 (81.6%) were from inpatient wards (IPD), followed by 417 (9.8%) from intensive care units (ICUs) and 365 (8.6%) from OPDs. The highest proportion of culture-positive cases was observed in the 19-30 year age group 835 (19.7%), whereas the lowest was in the 13-18 year age group 116 (2.7%), as shown in [Table/Fig-1].

Age (years)	n (%)
0-12	370 (8.7)
13-18	116 (2.7)
19-30	835 (19.7)
31-40	822 (19.4)
41-50	756 (17.8)
51-60	530 (12.5)
>60	819 (19.3)

[Table/Fig-1]: Age-wise distribution of UTI cases across different age groups (n=4248).

The monthly distribution of UTI cases over the five-year study period is shown in [Table/Fig-2]. After adjusting for year, negative binomial regression revealed no statistically significant variation in UTI case counts across months (p-value=0.888), suggesting no consistent monthly pattern over the five-year study period. After adjusting for month, UTI case counts varied significantly across the study years (p-value<0.001), with higher case counts continuing after 2022.

Month	2020	2021	2022	2023	2024
Jan	96 (15.1%)	43 (7.8%)	45 (4.8%)	128 (12.1%)	89 (8.3%)
Feb	74 (11.7%)	41 (7.5%)	52 (5.5%)	106 (10.0%)	94 (8.8%)
Mar	67 (10.6%)	53 (9.6%)	95 (10.1%)	101 (9.5%)	100 (9.4%)
Apr	45 (7.1%)	22 (4%)	107 (11.4%)	111 (10.5%)	106 (9.9%)
May	28 (4.4%)	42 (7.6%)	63 (6.7%)	103 (9.7%)	106 (9.9%)
Jun	45 (7.1%)	3 (0.5%)	80 (8.5%)	97 (9.2%)	94 (8.8%)
Jul	20 (3.2%)	73 (13.3%)	45 (4.8%)	67 (6.3%)	101 (9.5%)
Aug	42 (6.6%)	63 (11.5%)	77 (8.2%)	71 (6.7%)	91 (8.5%)
Sep	62 (9.8%)	55 (10.0%)	58 (6.2%)	46 (4.3%)	71 (6.7%)
Oct	79 (12.5%)	45 (8.2%)	88 (9.4%)	91 (8.6%)	78 (7.3%)
Nov	44 (6.9%)	58 (10.5%)	106 (11.3%)	73 (6.9%)	62 (5.8%)
Dec	32 (5%)	52 (9.5%)	123 (13.1%)	64 (6.0%)	75 (7.0%)
Total (n)	634	550	939	1058	1067
Statistical analysis					
Variable			**LR χ^2**	**df**	**p-value**
Month, adjusted for year			5.77	11	0.888
Year, adjusted for month			29.87	4	<0.001

[Table/Fig-2]: Monthly distribution of UTI cases over five years (n=4,248). Negative binomial regression was used to assess monthly variation in UTI case counts after adjustment for study year; LR: likelihood-ratio; df: degrees of freedom. A p-value <0.05 was considered statistically significant.

A total of 4,248 culture-positive isolates were recovered, of which 3229 (76.0%) were Gram-negative bacilli, and 1019 (24.0%) were Gram-positive cocci. The most common uropathogen was *E. coli*, isolated in 1834 (43.2%) of cases. *K. pneumoniae* was the second most common pathogen, isolated in 876 (20.6%) cases. Among Gram-positive organisms, enterococci (*Enterococcus* spp., *E. faecium*, and *E. faecalis*) collectively accounted for 891 (21.0%) isolates and were the predominant Gram-positive pathogens. Other commonly isolated organisms included *P. aeruginosa* 269 (6.3%) and *S. aureus* 68 (1.6%) [Table/Fig-3].

Among the Gram-negative isolates, *E. coli* showed the highest resistance to ampicillin 1680 (92.3%), cefotaxime 1656 (91.9%), ceftriaxone 1606 (89.6%), and norfloxacin 1372 (87.8%). In contrast, colistin resistance remained low at 4 (0.2%), while nitrofurantoin resistance in *E. coli* was comparatively low at 288 (23.3%) compared with other GNB isolates [Table/Fig-4].

Gram negative organisms	Total isolates	Gram positive organisms	Total isolates
Escherichia coli	1834 (43.2%)	*Enterococcus* spp.*	439 (10.3%)
Klebsiella pneumoniae	876 (20.6%)	*Enterococcus faecalis*	297 (7%)
Klebsiella oxytoca	33 (0.8%)	*Enterococcus faecium*	155 (3.6%)
Klebsiella aerogenes	3 (0.1%)	*Staphylococcus aureus*	68 (1.6%)
Pseudomonas aeruginosa	269 (6.3%)	SOSA#	38 (0.9%)
Acinetobacter baumannii	120 (2.8%)	*Streptococcus* spp.	22 (0.5%)
Enterobacter spp.	14 (0.3%)		
Citrobacter spp.	17 (0.4%)		
Citrobacter koseri	17 (0.4%)		
Proteus mirabilis	18 (0.4%)		
Proteus vulgaris	14 (0.3%)		
Morganella morganii	2 (0.1%)		
Providencia spp.	4 (0.1%)		
Non-fermenting GNB	8 (0.2%)		
Total	3229	Total	1019

[Table/Fig-3]: Distribution of gram-negative bacilli and gram-positive cocci isolated from urine cultures during the study period (n=4248).
* *Enterococcus* speciation was performed from 2023 onwards at our facility. Hence, the *Enterococcus* spp. isolate total is till 2022. From 2023 onwards, *Enterococcus* species data is given individually. # SOSA: Staphylococci other than *Staphylococcus aureus*

Among the Gram-positive isolates, *Enterococcus* demonstrated high resistance to erythromycin 384 (88.9%), clindamycin 66 (84.6%), ciprofloxacin 523 (78.2%), norfloxacin 410 (77.7%), and High-level gentamicin 526 (65.7%). In contrast, resistance to vancomycin 42 (5.3%), teicoplanin 88 (11.4%), nitrofurantoin 163 (23.2%), and doxycycline 56 (21.2%) remained comparatively low. *S. aureus* exhibited high resistance to penicillin G 58 (93.5%), while no vancomycin resistance was detected [Table/Fig-5].

The Cochran-Armitage trend test showed significant increasing resistance trends in *E. coli* to ampicillin (p-value=0.0008), Cefuroxime (p-value<0.0001), Ceftriaxone (p-value<0.0001), Cefotaxime (p-value<0.0001), and Imipenem (p-value<0.0001). In contrast, significant decreasing resistance trends were observed for amoxicillin-clavulanic acid, piperacillin-tazobactam, cefepime, trimethoprim-sulfamethoxazole, gentamicin, amikacin, tetracycline, and norfloxacin, whereas ceftazidime, meropenem, ciprofloxacin, nitrofurantoin, and colistin showed no significant temporal trend [Table/Fig-6].

Among *K. pneumoniae* isolates, the trend test demonstrated significant declines in resistance to Cefepime (p-value<0.0001), Trimethoprim–Sulfamethoxazole (p-value<0.0001), and Tetracycline (p-value<0.0001). Significant trends were also observed for Amoxicillin-Clavulanic acid (p-value=0.0324), Cefuroxime (p-value<0.0001), and Meropenem (p-value=0.0265). No significant temporal trends were observed for piperacillin-tazobactam, ceftriaxone, cefotaxime, imipenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, nitrofurantoin, or colistin [Table/Fig-7].

Resistance phenotypes revealed increasing trend during the study period. In 2024, ESBL production was very common in *E. coli* 305 (77.6%) and *K. pneumoniae* 157 (96.3%). Carbapenem-Resistant Enterobacterales (CRE) and MDR were also prevalent, with *K. pneumoniae* being the most common pathogen. Conversely, Pan-Drug Resistance (PDR) remained less frequent in our geographical setting.

Among *Enterococcus* isolates, significant linear increases in resistance were observed for ampicillin (p-value=0.0229), ciprofloxacin (p-value<0.0001), norfloxacin (p-value=0.0005), tetracycline (p-value<0.0001), and teicoplanin (p-value=0.0101). No significant temporal trends were observed for high-level gentamicin, nitrofurantoin, vancomycin, or linezolid. Fosfomycin was tested only in *E. faecalis* isolates from 2023 onwards; therefore, trend analysis was not performed [Table/Fig-8].

Antibiotic name	*E. coli*		*K. pneumoniae*		*P. aeruginosa*		*A. baumannii*	
	Total tested (n)	Resistant isolates n (%)	Total tested (n)	Resistant isolates n (%)	Total tested (n)	Resistant isolates n (%)	Total tested (n)	Resistant isolates n (%)
Ampicillin	1821	1680 (92.3)	IR	-	IR	-	IR	-
Amoxicillin/ Clavulanic acid	1536	1102 (71.7)	604	421 (69.7)	IR	-	IR	-
Piperacillin/ Tazobactam	1528	944 (61.7)	797	502 (63.0)	239	0	108	57 (52.8)
Cefuroxime	1762	1603 (90.9)	173	133 (76.9)	247	79 (32.0)	18	16 (88.9)
Ceftazidime	1642	1330 (80.9)	517	400 (77.4)	195	11 (5.6)	97	66 (68.0)
Ceftriaxone	1793	1606 (89.6)	234	191 (81.6)	IR	-	25	21 (84.0)
Cefotaxime	1802	1656 (91.9)	686	576 (84.0)	IR	-	79	53 (67.1)
Cefepime	1706	1225 (71.8)	503	401 (79.7)	56	43 (76.8)	49	32 (65.3)
Aztreonam	1675	1168 (69.7)	161	125 (77.6)	98	53 (54.1)	IR	-
Imipenem	1584	772 (48.7)	566	264 (46.6)	216	94 (43.5)	80	46 (57.5)
Meropenem	1269	660 (47.2)	833	421 (50.5)	169	75 (44.4)	111	57 (51.4)
Amikacin	1023	433 (42.32)	625	313 (50.1)	255	104 (40.8)	74	44 (59.5)
Gentamicin	1265	695 (54.94)	687	350 (50.9)	196	94 (48.0)	101	53 (52.5)
Ciprofloxacin	1723	1486 (86.2)	675	512 (75.9)	211	102 (48.3)	83	40 (48.2)
Norfloxacin	1563	1372 (87.8)	631	449 (71.2)	3	0	72	0 (0.0)
Trimethoprim/ Sulfamethoxazole	1352	1060 (78.4)	647	443 (68.5)	IR	-	99	55 (55.6)
Colistin[#]	1800	4 (0.2)	232	6 (2.5)	83	0	45	0
Nitrofurantoin	1236	288 (23.3)	823	459 (55.8)	184	136 (73.9)	93	63 (67.7)
Doxycycline	1548	345 (22.2)	74	14 (18.9)	10	4 (40.0)	12	5 (41.7)
Minocycline	NT	-	25	5 (20.0)	NT	-	54	19 (35.2)
Tetracycline	1654	999 (60.3)	572	318 (55.6)	IR	-	43	16 (37.2)

[Table/Fig-4]: Overall antimicrobial resistance profile of major Gram-negative uropathogens isolated during the five-year study period (2020-2024).
NT: Not tested; IR: Intrinsic resistance; [#](Colistin testing done via BMD)

Antibiotic name	*Enterococcus* spp		*Staphylococcus aureus*	
	Total samples tested (n)	Resistant isolates n (%)	Total samples tested (n)	Resistant isolates n (%)
Penicillin G	281	186 (66.2)	62	58 (93.5)
Ampicillin	612	412 (67.3)	NT	NT
Amoxicillin/Clavulanic acid	10	6 (60.0)	NT	NT
Piperacillin/Tazobactam	3	1 (33.3)	NT	NT
Ceftriaxone	3	3 (100.0)	NT	NT
Cefotaxime	12	10 (83.3)	NT	NT
Cefepime	3	3 (100.0)	NT	NT
Cefoxitin	64	53 (82.8)	93	69 (74.2)
Aztreonam	2	2 (100.0)	NT	NT
Meropenem	1	0	NT	NT
High-level Gentamicin	801	526 (65.7)	NT	NT
Gentamicin	NT	NT	44	14 (31.8)
Ciprofloxacin	669	523 (78.2)	92	64 (69.6)
Norfloxacin	528	410 (77.7)	52	32 (61.5)
Trimethoprim/ Sulfamethoxazole	185	60 (32.4)	65	20 (30.8)
Clindamycin	78	66 (84.6)	76	39 (51.3)
Erythromycin	432	384 (88.9)	64	39 (60.9)
Nitrofurantoin	702	163 (23.2)	82	9 (11.0)
Vancomycin	792	42 (5.3)	62	0
Teicoplanin	772	88 (11.4)	61	9 (14.8)
Doxycycline	264	56 (21.2)	38	9 (23.7)
Tetracycline	523	309 (59.1)	33	6 (18.2)

[Table/Fig-5]: Overall antimicrobial resistance profile of major gram-positive uropathogens isolated during the five-year study period (2020–2024).
NT: Not tested

Antibiotic	2020 (n=309)	2021 (n=253)	2022 (n=439)	2023 (n=440)	2024 (n=393)	χ^2 trend	p-value
Ampicillin	303 (98%)	243 (96%)	399 (91%)	409 (93%)	362 (92%)	11.2247	0.00081
Amoxicillin-clavulanic acid	226 (73%)	142 (56%)	215 (49%)	251 (57%)	236 (60%)	7.315	0.00684
Piperacillin-tazobactam	201 (65%)	119 (47%)	189 (43%)	216 (49%)	200 (51%)	8.2332	0.00411

Cefuroxime	201 (65%)	164 (65%)	369 (84%)	370 (84%)	330 (84%)	59.3774	<0.0001
Ceftazidime	232 (75%)	185 (73%)	320 (73%)	321 (73%)	287 (73%)	0.3067	0.57974
Ceftriaxone	235 (76%)	202 (80%)	369 (84%)	387 (88%)	350 (89%)	29.534	<0.0001
Cefotaxime	247 (80%)	210 (83%)	373 (85%)	387 (88%)	354 (90%)	17.9144	<0.0001
Cefepime	256 (83%)	200 (79%)	320 (73%)	308 (70%)	267 (68%)	25.9726	<0.0001
Imipenem	114 (37%)	63 (25%)	101 (23%)	167 (38%)	189 (48%)	22.1992	<0.0001
Meropenem	102 (33%)	51 (20%)	110 (25%)	150 (34%)	122 (31%)	1.7713	0.18322
Amikacin	105 (34%)	56 (22%)	176 (40%)	106 (24%)	94 (24%)	8.1152	0.00439
Gentamicin	158 (51%)	106 (42%)	154 (35%)	172 (39%)	161 (41%)	6.4858	0.01087
Ciprofloxacin	260 (84%)	200 (79%)	356 (81%)	339 (77%)	322 (82%)	0.9744	0.32359
Norfloxacin	272 (88%)	200 (79%)	351 (80%)	365 (83%)	307 (78%)	5.7184	0.01679
Trimethoprim-sulfamethoxazole	244 (79%)	164 (65%)	263 (60%)	273 (62%)	228 (58%)	28.366	<0.0001
Nitrofurantoin	65 (21%)	33 (13%)	79 (18%)	62 (14%)	63 (16%)	2.3709	0.12361
Tetracycline	201 (65%)	134 (53%)	250 (57%)	246 (56%)	212 (54%)	5.4747	0.01929
Colistin	0	0	0	4 (0.9%)	0	1.393	0.23789

[Table/Fig-6]: Temporal trends in antimicrobial resistance among *E. coli* isolates (2020-2024).
χ^2 trend- Cochran-Armitage chi-square test for linear trend; Sig.- Statistical significance; df- Degrees of freedom (df=1 for all rows); Values are presented as resistant isolates, n (%), with percentages calculated using the total number of *E. coli* isolates tested for each antimicrobial in the respective year. A p-value <0.05 was considered statistically significant.

Antibiotic	2020 (n=154)	2021 (n=158)	2022 (n=205)	2023 (n=196)	2024 (n=163)	χ^2 trend	p-value
Amoxicillin-clavulanic acid	132 (86%)	96 (61%)	135 (66%)	127 (65%)	116 (71%)	4.5765	0.03241
Piperacillin-tazobactam	95 (62%)	95 (60%)	131 (64%)	123 (63%)	106 (65%)	0.5824	0.44538
Cefuroxime	129 (84%)	121 (76.6%)	164 (80%)	159 (81%)	134 (82%)	18.1383	<0.0001
Ceftriaxone	154 (100%)	131 (83%)	164 (80%)	172 (88%)	145 (89%)	3.7947	0.05142
Cefotaxime	132 (86%)	131 (83%)	166 (81%)	159 (81%)	130 (80%)	2.0045	0.15683
Cefepime	136 (88%)	133 (84%)	160 (78%)	149 (76%)	117 (72%)	16.6331	<0.0001
Imipenem	74 (48%)	58 (37%)	86 (42%)	92 (47%)	80 (49%)	1.1104	0.29199
Meropenem	89 (58%)	47 (30%)	103 (50%)	112 (57%)	93 (57%)	4.9208	0.02653
Amikacin	94 (61%)	58 (37%)	107 (52%)	108 (55%)	86 (53%)	0.0629	0.802
Gentamicin	83 (54%)	79 (50%)	107 (52%)	100 (51%)	73 (45%)	1.8362	0.1754
Ciprofloxacin	123 (80%)	122 (77%)	158 (77%)	149 (76%)	127 (78%)	0.2505	0.61673
Norfloxacin	108 (70%)	114 (72%)	152 (74%)	139 (71%)	106 (65%)	1.0279	0.31065
Trimethoprim–Sulfamethoxazole	128 (83%)	114 (72%)	133 (65%)	123 (63%)	93 (57%)	27.4682	<0.0001
Nitrofurantoin	94 (61%)	79 (50%)	125 (61%)	104 (53%)	86 (53%)	1.2338	0.26668
Tetracycline	89 (58%)	88 (56%)	113 (55%)	80 (41%)	65 (40%)	17.3605	<0.0001
Colistin	0	6 (4%)	0	0	0	3.7015	0.05437

[Table/Fig-7]: Temporal trends in antimicrobial resistance among *K. pneumoniae* isolates (2020-2024).
χ^2 trend- Cochran-Armitage Chi-square test for linear trend; Sig.- Statistical significance; df- Degrees of freedom (df=1 for all rows); Values are presented as resistant isolates, n (%), with percentages calculated using the total number of *K. pneumoniae* isolates tested for each antimicrobial in the respective year. A p-value <0.05 was considered statistically significant.

Antibiotic	2020 (n=105)	2021 (n=87)	2022 (n=183)	2023 (n=200)	2024 (n=316)	χ^2 trend	p-value
Ampicillin	83 (79.0%)	50 (58%)	128 (70%)	126 (63%)	199 (63%)	5.1801	0.02285
High level gentamicin	67 (63.8%)	32 (37%)	117 (64%)	108 (54%)	202 (64%)	2.2805	0.13101
Ciprofloxacin	80 (76.2%)	59 (68%)	126 (69%)	160 (80%)	281 (89%)	23.0423	<0.0001
Fosfomycin*	-	-	-	36 (28.6%)	45 (35.4%)	NA	NA
Linezolid	0	0	0	0	0	0	1
Norfloxacin	74 (70.5%)	61 (70%)	137 (75%)	160 (80%)	262 (83%)	11.9584	0.00054
Nitrofurantoin	18 (17.1%)	20 (23%)	40 (22%)	42 (21%)	85 (27%)	3.7056	0.05423
Tetracycline	52 (49.5%)	36 (41%)	97 (53%)	120 (60%)	243 (77%)	48.0186	<0.0001
Teicoplanin	3 (2.9%)	4 (5%)	22 (12%)	24 (12%)	35 (11%)	6.6122	0.01013
Vancomycin	3 (2.9%)	5 (6%)	11 (6%)	10 (5%)	13 (4%)	0.0006	0.97972

[Table/Fig-8]: Temporal trends in antimicrobial resistance among *enterococcus* isolates (2020-2024).
χ^2 trend- Cochran-Armitage chi-square test for linear trend; Sig.: Statistical significance; df- Degrees of freedom (df=1 for all rows). Values are presented as resistant isolates, n (%), with percentages calculated using the total number of *Enterococcus* isolates tested for each antimicrobial in the respective year. A p-value <0.05 was considered statistically significant. Fosfomycin was tested only in *E. faecalis* isolates (2023, n=126; 2024, n=127); therefore, no trend analysis was performed. A p-value <0.05 was considered statistically significant.

DISCUSSION

In the present study, the majority of culture-positive isolates with the continued predominance of Gram-negative bacilli, particularly *E. coli*, with females accounting for 2219 (52.2%) of culture-positive UTI cases, consistent with findings of female predominance in UTI cases as reported by Kumar KGS et al., from South India 128 (71.1%) and Narain T et al., from Patna 127 (63.5%) [5,17] were obtained from hospitalised patients, predominantly from the IPD setting. As compared to Karuna T et al., who reported a predominance of outpatient UTI cases [18]. This variation may be attributed to differences in clinical practice.

The present study showed a steady rise in culture-positive UTI cases over the five-year study period, from 634 (14.9%) in 2020 to 1067 (25.1%) in 2024, with a significant increase post-2021. Negative binomial regression confirmed a significant year-wise increase after adjusting for monthly variation, while no consistent seasonal pattern was observed.

The predominant uropathogens were *E. coli* 1834 (43.2%) and *K. pneumoniae* 876 (20.6%). These results align with study from Bihar by Narain T et al., [17]. The antimicrobial resistance profile of the present study showed significant resistance among Gram-negative and Gram-positive uropathogens to commonly used empirical antibiotics. The highest resistance in Gram-negative isolates was observed against ampicillin 1680 (92.3%), cefotaxime 2285 (89.0%), ceftriaxone 1818 (88.6%), ciprofloxacin 2140 (79.5%), and norfloxacin 1821 (80.3%), as reported by Karuna T et al., and Narain T et al., a continued decline in the efficacy of β- lactams and fluoroquinolones [17,18]. In contrast, Dunne MW et al., from the United States reported considerably lower norfloxacin resistance, with only 1071 (20.0%) resistant isolates among outpatient urinary isolates [19]. Moreover, the current study showed a significant burden of Gram-positive cocci, 1019 (24.0%), mainly *Enterococcus* spp. 439 (10.4%), suggesting a rather diverse uropathogen profile, coinciding with studies of Behairy MA et al., reflecting the changing microbiological spectrum of complicated and healthcare-associated UTI [20]. Furthermore, the presence of other uropathogens in the current study, such as *P. aeruginosa* 269 (6.3%), *A. baumannii* 120 (2.8%), and *S. aureus* 68 (1.6%), highlights a growing challenge for clinicians.

High resistance was observed among Gram-positive isolates to ciprofloxacin 523 (78.2%) and norfloxacin 410 (77.7%), gentamicin 526 (65. 7%) for *Enterococcus* isolates, exceeding the prevalence reported by Arundathi HA et al., 27 (42.0%) [21]. Similarly, Shams Abadi MS et al., reported a pooled High-level gentamicin resistance prevalence of 464 (64.8%) among *E. faecium* and 920 (42.2%) among *E. faecalis* [22].

Whereas linezolid 0 (0.0%), vancomycin 42 (5.3%), and teicoplanin 88 (11.4%) maintained good activity. Behairy MA et al., reported similar results, showing preserved susceptibility of *Enterococcus* spp. to vancomycin and linezolid, despite high resistance to fluoroquinolones in hospitalised patients [20]. Nitrofurantoin had relatively low resistance in both Gram-negative isolates 1308 (30.8%) and Gram-positive isolates 163 (23.2%), which validates its ongoing role as an effective first-line agent for uncomplicated lower UTIs caused by susceptible organisms.

Analysis of the temporal trend showed a changing pattern of antimicrobial resistance among the major uropathogens over the five-year study period. Resistance to cefuroxime increased from 65.0% in 2020 to 84.0% in 2024; to ceftriaxone from 76.0% to 89.0%; and to cefotaxime from 80.0% to 90.0%. Imipenem resistance increased significantly from 37.0% to 48.0%, and trimethoprim-sulfamethoxazole resistance decreased significantly from 79.0% to 58.0%. Karuna T et al., and Narain T et al., reported similar resistance patterns, with high resistance to cephalosporins and fluoroquinolones among uropathogens [17,18]. *K. pneumoniae* showed significant decreases in resistance to cefepime 88.0% to 72.0%, trimethoprim-sulfamethoxazole 83.0% to 57.0% and tetracycline 58.0% to 40.0%. Resistance to ciprofloxacin, norfloxacin, and tetracycline increased significantly in *Enterococcus* spp., from 76.2% to 89.0%, 70.5% to 83.0%, and 49.5% to 77.0%, respectively, whereas resistance to linezolid and vancomycin remained low throughout the study period. These results emphasise the need for continued institutional antimicrobial resistance surveillance to guide empiric therapy and bolster antimicrobial stewardship programs.

The high prevalence of multidrug-resistant phenotypes observed in the present study further highlights the growing problem of antimicrobial resistance among uropathogens. Similar observations have been reported by Shree R et al., who reported a high prevalence of CRE, and by Behairy MA et al., who reported a significant burden of multidrug-resistant uropathogens among hospitalised patients [20,23]. Conversely, earlier Indian study by Narain T et al., demonstrated a significantly lower level of ESBL producers at 83 (41.5%) CRE at 8 (4.0%) among uropathogens, indicating a gradual rise in resistance in recent years [17]. The much higher resistance burden observed in *K. pneumoniae* underscores its increasing importance as a healthcare-associated pathogen and the need for ongoing surveillance, rigorous infection prevention practices, and antimicrobial stewardship programs.

The clinical impact of inappropriate empirical therapy was illustrated by Dunne MW et al., who showed that patients treated with empirical antibiotics to which the causative organism was resistant were almost twice as likely to require hospitalisation [19]. Moreover, Baimakhanova B et al., emphasised the potential of rapid molecular diagnostic methods, including multiplex PCR and next-generation sequencing, for early detection of ESBL, CRE, MDR and PDR-producing uropathogens, antimicrobial resistance and optimisation of targeted treatment optimisation [24]. Until such approaches are widely available, ongoing institutional surveillance, regular updating of hospital-specific antibiograms, implementation of robust antimicrobial stewardship programmes and stringent infection prevention measures will remain essential to optimise empirical therapy and prevent the emergence and spread of antimicrobial resistance.

Limitation(s)

As a retrospective, single-centre study, the findings may not be generalisable to other healthcare settings. Molecular characterisation of resistance determinants and genotypic confirmation of ESBL and carbapenemase production were not performed. In addition, clinical risk factors, prior antibiotic exposure, treatment outcomes, and patient follow-up were not evaluated. Furthermore, several less frequently isolated pathogens were represented by fewer than 100 isolates, limiting the robustness of organism-specific resistance analysis and the interpretation of temporal trends for these organisms. These data will serve as a baseline for future longitudinal surveillance to evaluate changes in antibiotic resistance in the region.

CONCLUSION(S)

This five-year longitudinal study underscores a substantial burden of UTIs, predominantly caused by Gram-negative bacilli such as *E. coli* and *K. pneumoniae*, with notable prevalence in inpatient settings. The findings reveal an alarming escalation in antimicrobial resistance, particularly the widespread emergence of ESBL-producers and MDR phenotypes that severely limit the efficacy of conventional β-lactams and fluoroquinolones. While carbapenems and aminoglycosides currently retain moderate effectiveness, the high rates of resistance to ciprofloxacin and cephalosporins necessitate a strategic shift towards more targeted therapies. Although nitrofurantoin for uncomplicated UTIs and linezolid and glycopeptides for enterococcal infections retain good activity, the progressive emergence of resistant phenotypes underscores the need for judicious antimicrobial use. Ultimately, these results highlight the imperative for robust antimicrobial stewardship and the implementation of hospital-specific antibiograms to combat the rising tide of PDR and CRE uropathogens.

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