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Enhancing antimicrobial management: A 5–year retrospective analysis of urinary pathogen susceptibility at a tertiary care centre from January 2018 to December 2022

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ABSTRACT

Objective: The study aims to collect data on prevalence and antibiotic susceptibility patterns of uropathogens in a hospital in India, and to analyze any changes that have occurred over the past 5 years.

Methods: This retrospective study was conducted over 5 years at Lady Hardinge Medical College & Associated Hospitals, New Delhi. Urine samples from patients who attended outpatient department or were admitted with diagnosis of urinary tract infection (UTI) were included in this study. Samples were cultured and tested to identify bacteria and determine antibiotic susceptibility using the Kirby-Bauer disc diffusion method.

Results: A total of number of 151395 urine specimens were collected from January 2018 to December 2022. The prevalence of UTI was 7.5% (11372/151395) with the majority of patients being aged above 40 years (19.3%). The prevalence was higher in females (12.7%) as compared to males (3.2%). *Escherichia coli* was the predominant pathogen, accounting for 59.8% of cases, followed by *Klebsiella* spp. at 18.9%, and *Enterococcus* spp. at 8.3%. Gram-negative bacteria accounted for 89.5% of the isolates and exhibited good susceptibility to aminoglycosides and piperacillin-tazobactam, but showed resistance to fluoroquinolones. Methicillin-resistant *Staphylococcus aureus* (MRSA) was present in 35% of *Staphylococcus aureus* isolates with high susceptibility to glycopeptides and nitrofurantoin.

Conclusions: The data suggest the need for careful selection of empirical therapy due to increasing resistance patterns. The stringent use of antimicrobial agents is pivotal to the control of antimicrobial resistance. Antibiotic susceptibility data contributes to tailoring the antibiotic stewardship programs to local needs.

KEYWORDS: Urinary tract infection; Extended-spectrum beta -lactamase; Resistance; Antibiotic susceptibility testing

1. Introduction

There has been an increase in the transmission of urinary tract infection (UTI) among men and women, with the higher incidence observed in women biologically[1]. Anatomical factors, such as a short urethra and fecal-perineal-urethral contamination, as well as certain physiological changes are responsible for the more frequent occurrence of UTI in females[2,3].

Infection of urinary tract are classified into two types: upper UTI (pyelonephritis) and lower UTI (cystitis). Symptoms of lower UTI include dysuria (burning sensation or pain while micturition) due to inflammation and irritatation of the urethra and urinary bladder lining, increased frequency of micturition, pain in the lower abdomen, an increased sensation of urine, mild fever, foul smell

Significance

In the recent years, there has been an increase in the resistance to antimicrobials used for treating urinary tract infections. Antimicrobial susceptibility test data may vary across different regions and time periods. This study provides insights into the patterns of antimicrobial susceptibility among pathogens causing urinary tract infection in our institution, which will significantly contribute to guiding empirical treatment choices, tailoring the antibiotic stewardship programs, and serving as an alert against the rise of multidrug-resistant strains.

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or blood in urine. Common symptoms in the upper UTI include high fever with chills, pain in waist on one side or back, nausea and vomiting[4].

The most common organisms involved in uncomplicated UTIs, where there are no functional or anatomical abnormalities in the urinary tract, no kidney function impairment, and no concomitant diseases predisposing to UTI or risk of developing serious complications, are usually Gram-negative bacteria such as *Escherichia* (*E.*) *coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* (*E.*) *aerogenes*, *Pseudomonas* (*P.*) *aeruginosa*, *Proteus vulgaris*, *Staphylococcus* (*S.*) *aureus*, and *Salmonella* spp.[5]. Uropathogenic *E. coli* is the leading causative organism in both uncomplicated and complicated UTI. The organisms causing complicated UTI include Gram-negative organisms such as *E. coli*, *Klebsiella* spp., *P. mirabilis*, *Providencia* (*P.*) *stuartii* and *Morganella* (*M.*) *morganii*, as well as Gram-positive organisms such as *S. aureus*, *Enterococcus* spp.[6].

Susceptibility patterns are used to describe likelihood that a particular antibiotic will be effective against a specific bacteria causing an infection. Physicians usually provide empirical therapy based on the observed susceptibility pattern in the area. Therefore, monitoring for changes in epidemiology and the resistance pattern of the organisms causing UTI is duly needed[7]. Various factors such as the type of UTI (complicated or uncomplicated), gender, age, and previous history of antibiotic therapy of each UTI patient should also be considered to gather correct data and monitor trends on prevalence and susceptibility patterns of causes of UTI [8].

The ideal selection of antibiotics should be based on the susceptibility pattern of the antibiotics in that geographic area. The retrospective study was conducted to find the organisms causing UTI and their susceptibility patterns.

2. Materials and methods

2.1. Study design and sample processing

The retrospective observational study was conducted in the Department of Microbiology, Lady Hardinge Medical College & Associated Hospitals, New Delhi, over a 5-years period from 1st January 2018 to 31st December 2022. A total of 151 395 samples from patients who attended outpatient department or were admitted with diagnosis of UTI were processed as per clinician's request during the study period. The patients with previous history of antibiotics were excluded from the study.

Urine samples collected were cultured within 1-2 h in the microbiology department on Cystine-Lactose-Electrolyte Deficient agar and incubated at 37°C for 24 h. A sterile 2.0 mm platinum wired calibrated loop was used for the culture. According to the standard protocol, the urine sample was considered positive for UTI if an organism was cultured at a concentration of $\geq 10^5$ CFU/mL or when an organism was cultured at a concentration of 10^4 CFU/mL and >5 pus cells per high-power field were observed on microscopic examination of the centrifuged urine[9]. The growth in most samples consisted of a single species although in some samples growth of two species like *E. coli* and *Enterococcus* spp. were also observed in significant numbers. The identification of the isolates was done by standard biochemical tests[5,10,11].

2.2. Antibiotic susceptibility testing

Isolates were tested for antimicrobial susceptibility by the standard Kirby Bauer's disc diffusion method. After 24 h the inhibition zones were measured and interpreted as per the recommendations of clinical and laboratory standards[12]. The antibiotic discs used were: 1) For Gram-negative organisms: Ampicillin (AMP-10 µg), amoxy-clavulanate (AMC-30 µg), gentamicin (GEN-10 µg), amikacin (AMK-30 µg), ciprofloxacin (CIP-5 µg), ceftazidime (CAZ-30 µg), ceftazolin (CZO-30 µg), cefotaxime (CTX-30 µg), norfloxacin (NOR-10 µg), cotrimoxazole (SXT-25 µg), nitrofurantoin (NIT-300 µg), tetracycline (TCY-30 µg), doxycycline (DOX-30 µg), minocycline (MNO-30 µg), piperacillin-Tazobactam (TZP-100/10 µg), imipenem (IMI-10 µg), meropenem (MEM-10 µg), colistin (COL-10 µg). 2) For Gram-positive organisms: penicillin (PEN-10 µg), erythromycin (ERY-15 µg), vancomycin (VAN-30 µg), teicoplanin (TEC-30 µg), linezolid (LNZ-30 µg), gentamicin (high-level gentamicin for Enterococcus-120 µg) and cefoxitin (FOX-30 µg). Ertapenem is intrinsically resistant to *Acinetobacter* (*A.*) *baumannii* and *P. aeruginosa* so these antibiotics were not used for those uropathogens. Moreover, as *P. aeruginosa* is intrinsically resistant to tetracycline, cefotaxime, and *Klebsiella* spp. is also intrinsically resistant to ampicillin, these antibiotics were not reported.

2.3. Extended–spectrum beta–lactamase detection

The phenotypic confirmation of extended-spectrum beta-lactamase (ESBL) was conducted by testing the strain against ceftazidime (CAZ) and ceftazidime/clavulanic acid. An inhibition zone diameter greater than 5 mm for ceftazidime/clavulanic acid compared to ceftazidime was considered indicative of ESBL production. *E. coli* ATCC 25922 was used as an ESBL-negative control strain, and *Klebsiella* spp. ATCC 700603 was used as an ESBL-positive control strain[13].

2.4. Statistical analysis

Data were entered in Microscoft excel and results were represented in graph and tables. Data were entered and analyzed using SPSS version 25.0 software, and results were presented in tables.

2.5. Ethical approval

The Institutional Ethics Committee of Lady Hardinge Medical College & Associated Hospitals approved the study with reference number LHMC/IEC/1/2024/90 dated 12 September, 2024.

3. Results

The prevalence of UTI in this study was found to be 7.5% (11 372/151 395). As shown in Table 1, UTI was observed to be higher in female patients (12.7%) than in males (3.2%). Among different age groups, the highest prevalence of UTI was observed among the older age group (>40 years) at a rate of 19.3%, followed by the 19-29 years age group (6.0%), 30-40 years age group (3.9%) and individuals 18 years old or younger (3.9%). *E. coli* was found to be the predominant organism accounting for 59.8% (6 800/11 372), followed by *Klebsiella* spp. at 18.9% (2 149/11 372), *Enterococcus* spp. at 8.3% (943/11 372), *A. baumannii* at 3.9% (443/11 372), *P. aeruginosa* at 2.6% (296/11 372) and *S. aureus* at 1.5% (170/11 372), *Enterobacter* spp. (1.5%), *Proteus* spp. (1.4%), *Citrobacter* spp. (1.3%), *S. saprophyticus* (0.7%) and *Serratia* spp. (0.1%).

Most Gram-negative uropathogens, including *E. coli*, *Klebsiella* spp., and *P. aeruginosa*, were found to be susceptible to aminoglycosides. The sensitivity to gentamicin ranged from 65% to 80%, while the sensitivity to amikacin ranged from 67% to 83%. In contrast, *A. baumannii* exhibited significantly lower sensitivity, with only 33% susceptibility to amikacin. Reasonably good sensitivity profiles were also observed against all the Gram-negative bacteria isolated to antibiotics like piperacillin-tazobactam (66%-80%) and meropenem (57%-75%). Among fluroquinolones, higher resistance was observed for ciprofloxacin, with sensitivity only at 21% for *E. coli* and 43% for *Klebsiella* spp. For norfloxacin, the sensitivity of *Klebsiella* spp. (63%) was better than that of *E. coli* (43%). Nitrofurantoin was found to be effective against *E. coli* (85%) but had low sensitivity against *Klebsiella* spp. (47%), *P. aeruginosa* (10%) and *A. baumannii* (22%). *Pseudomonas* spp. showed good susceptibility profile for ciprofloxacin (69%) and norfloxacin (71%). *A. baumannii* showed excellent susceptibility profile for doxycycline (81%) and minocycline (80%). Colistin was observed to be 100% sensitive against all the Gram-negative organisms isolated (Table 2).

In Gram-positive cocci, it was observed that *S. aureus* and *Enterococcus* spp. showed good susceptibility to nitrofurantoin (92% and 80%, respectively), glycopeptides (ranging from 89% to 100%) and linezolid (100%). Additionally, *S. aureus* showed good susceptibility to aminoglycosides (71% for amikacin and 75% for gentamicin, respectively), doxycycline (82%), and minocycline (100%). However, a poor susceptibility was seen to fluoroquinolones (22% for ciprofloxacin and 18 % for norfloxacin). Majority of *S. aureus* (75%) and *Enterococcus* spp. (91%) isolates were resistant to erythromycin. Thirty-five percent of the *S. aureus* isolates were Methicillin Resistant *Staphylococcus Aureus* (MRSA) with the least susceptibility to penicillin, fluoroquinolones and erythromycin, and highest susceptibility to glycopeptides, nitrofurantoin and minocycline. Six percent of the *Enterococcus* spp. were vancomycin resistant (Table 3). The overall 58.0% ESBL positive organisms were observed including 61% *E. coli* and 72% *Klebsiella spp*. Interestingly, there appears to have been no significant change in the susceptibility of the two most common uropathogens (*E. coli* and *Klebsiella* spp.) to most of the antibiotics included in this study over the past 5 years. A slight improvement in ciprofloxacin sensitivity to *E. coli* observed, with a decline in the sensitivity of both *E. coli* and *Klebsiella* spp. to nitrofurantoin (Table 4). Additionally, there was a steady increase in ESBL positivity for *E. coli* over the years (Figure 1).

Table 1. Gender-wise and age-wise distribution of UTI (n=151 395).

Variables	Tested	Positive (>10^5 CFU/mL)	Percent (%)
Sex			
Male	83 115	2 700	3.3
Female	68 280	8 672	12.7
Age groups			
< 18 years	34 460	1 333	3.9
19-29 years	48 921	2 953	6.0
30-40 years	39 276	1 550	3.9
>40 years	28 738	5 536	19.3

CFU: Colony forming units; UTI: Urinary tract infection.

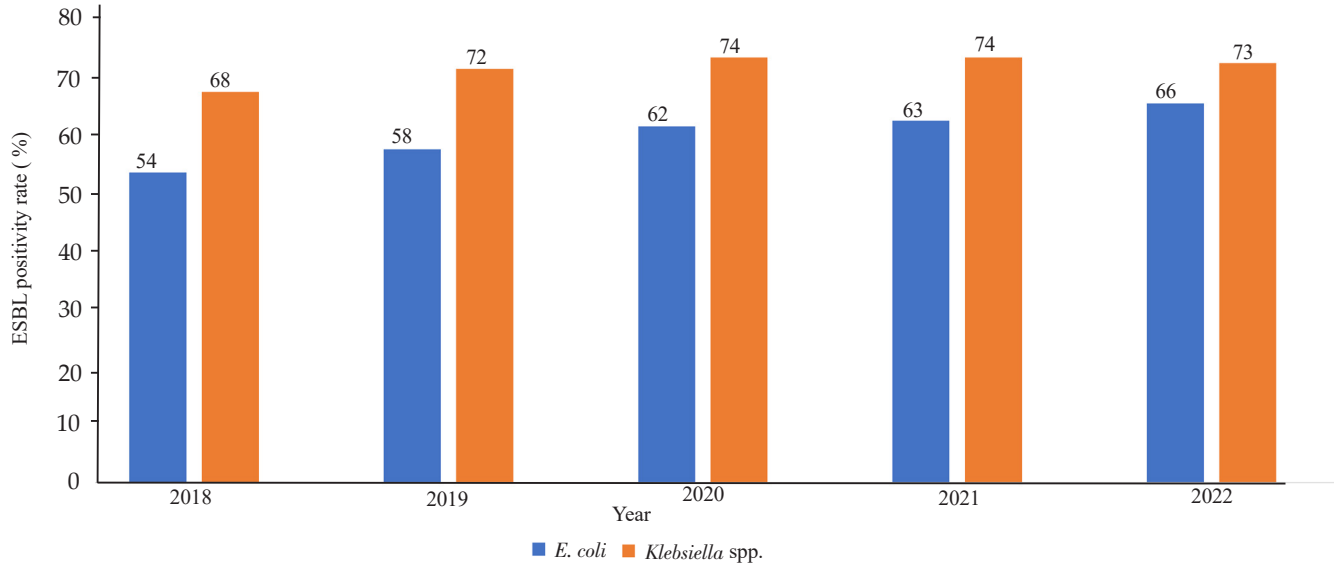

Figure 1. Trends in extended spectrum beta-lactamase (ESBL) positivity over the study period for most common uropathogens.

Table 2. Distribution of predominant Gram-negative organisms susceptibility pattern in UTI.

Gram-negative organism	Percent (%)	Antibiotic sensitivity pattern (%)																		
		AMP	AMC	CZO	CAZ	CTX	TZP	CIP	NOR	GEN	AMK	NIT	SXT	TCY	DOX	MIN	IMI	MEM	ERT	COL
E. coli	59.8	9	0	31	34	27	76	21	43	76	83	85	38	49	51	62	56	75	51	100
Klebsiella spp.	18.9	-	46	38	43	41	66	43	63	78	67	47	50	65	63	54	60	66	61	100
P. aeruginosa	2.6	-	-	6	66	-	80	69	71	80	77	10	23	-	0	33	56	68	-	100
A. baumanni	3.9	-	-	6	46	18	74	55	0	65	33	22	49	70	81	80	61	57	-	100

Note: ampicillin (AMP), amoxy-clavulanate (AMC), ceftazolin (CZO), ceftazidime (CAZ), cefotaxime (CTX), piperacillin-tazobactam (TZP), ciprofloxacin (CIP), norfloxacin (NOR), gentamicin (GEN), amikacin (AMK), nitrofurantoin (NIT), cotrimoxazole (SXT), tetracycline (TCY), doxycycline (DOX), minocycline (MIN), imipenem (IMI), meropenem (MEM), Ertapenem (ERT), colistin (COL).

Table 3. Distribution of predominant Gram-positive organisms susceptibility pattern in UTI.

Gram-positive organism	Percent (%)	Antibiotic sensitivity pattern (%)															
		PEN	CIP	NOR	AMK	GEN	ERY	NIT	SXT	CLI	FOX	TCY	DOX	MIN	TEC	VAN	LNZ
S. aureus	1.5*	4	22	18	71	75	25	92	55	60	65	68	82	100	90	100	100
Enterococcus spp.	8.3	59	22	18	25	(HLG)48	9	80	60	-	-	23	39	7	89	94	100

Note: penicillin (PEN), ciprofloxacin (CIP), norfloxacin (NOR), amikacin (AMK), gentamicin (GEN), erythromycin (ERY), nitrofurantoin (NIT), cotrimoxazole (SXT), clindamycin (CLI), cefoxitin (FOX), tetracycline (TCY), doxycycline (DOX), minocycline (MIN), teicoplanin (TEC), vancomycin (VAN), linezolid (LNZ), HLG (high-level gentamicin for *Enterococcus* spp.). *S. aureus* accounted for 1.5% of isolates, with 35% of these being methicillin-resistant *S. aureus* (MRSA).

Table 4. Trends of susceptibility over 5 years for predominant Gram-negative uropathogens.

	Gram-negtive organism	Antibiotic sensitivity pattern (%)																
		CZO	CAZ	CTX	TZP	CIP	NOR	GEN	AMK	NIT	SXT	TCY	DOX	MIN	IMI	MEM	ERT	COL
2018	*E. coli*	38	38	25	72	20	44	75	86	89	49	52	55	70	45	75	56	100
	Klebsiella spp.	40	36	45	70	42	64	74	72	55	58	61	60	58	55	67	65	100
	P. aeruginosa	8	69	-	85	73	76	86	80	15	26	-	0	37	60	74	-	100
	A. baumannii	8	50	22	78	59	0	68	36	20	47	75	83	84	63	61	-	100
2019	*E. coli*	31	34	27	76	18	43	70	79	88	47	49	51	62	49	79	51	100
	Klebsiella spp.	38	43	41	66	40	65	72	69	52	49	65	63	54	49	59	61	100
	P. aeruginosa	6	66	-	80	69	71	80	77	10	23	-	0	33	56	68	-	100
	A. baumannii	6	46	18	74	55	0	65	33	22	49	70	81	80	61	57	-	100
2020	*E. coli*	31	34	27	76	21	43	76	83	85	38	49	51	62	56	75	51	100
	Klebsiella spp.	38	43	41	66	43	63	78	67	47	50	65	63	54	60	66	61	100
	P. aeruginosa	6	66	-	80	69	71	80	77	10	23	-	0	33	56	68	-	100
	A. baumannii	6	46	18	74	55	0	65	33	22	49	70	81	80	61	57	-	100
2021	*E. coli*	31	34	27	76	21	43	76	83	85	38	49	51	62	56	75	51	100
	Klebsiella spp.	38	43	41	66	43	63	78	67	47	50	65	63	54	60	66	61	100
	P. aeruginosa	6	66	-	80	69	71	80	77	10	23	-	0	33	56	68	-	100
	A. baumannii	6	46	18	74	55	0	65	33	22	49	70	81	80	61	57	-	100
2022	*E. coli*	31	34	27	76	21	43	76	83	85	38	49	51	62	56	75	51	100
	Klebsiella spp.	38	43	41	66	43	63	78	67	47	50	65	63	54	60	66	61	100
	P. aeruginosa	6	66	-	80	69	71	80	77	10	23	-	0	33	56	68	-	100
	A. baumannii	6	46	18	74	55	0	65	33	22	49	70	81	80	61	57	-	100

Note: ceftazolin (CZO), ceftazidime (CAZ), cefotaxime (CTX), piperacillin-tazobactam (TZP), ciprofloxacin (CIP), norfloxacin (NOR), gentamicin (GEN), amikacin (AMK), nitrofurantoin (NIT), cotrimoxazole (SXT), tetracycline (TCY), doxycycline (DOX), minocycline (MIN), imipenem (IMI), meropenem (MEM), colistin (COL).

4. Discussion

The prevalence of UTI in the present study was found to be 7.5%. This is similar to the study conducted in south India in 2011 (9.0%)[7] and higher than a study in Nigeria (4.0%) in 2013[14]. However, a study conducted north Delhi in 2014 found the prevalence to be higher at 15.6%[15]. UTI was observed to be more common in women compared to men, which is consistent with findings from various other studies[16,17]. The reason for frequent UTI in women has been correlated to close proximity of urethra to anus[2,3].

The prevalence of UTI was higher among older patients (>40 years, 19.3%) compared to the younger age group. Similar results were observed in studies conducted in India[18] and Japan[19]. This can be attributed to the progressive increase in the size of the prostrate gland leading to neurogenic bladder[20]. Among culture positive cases, a higher rate of infection was observed in the older agae group (19.3%) followed by 19-29 year age group (6.0%) , which is consistent with findings from other studies[7,21]. In addition to women in reproductive age, elderly menopausal women also show

a higher rate of UTI[7]. The higher prevalence of UTI in younger women is associated with pregnancy and the use of a diaphragm with spermicide[22].

Gram-negative organisms were found to be the predominant (89.5%) cause of UTI. Gram-positive cocci composed of 10.5% of the isolates. The most prevalent Gram-negative organism causing UTI was *E. coli* (59.8%). This result has high prevalence to other reports from Kuwait (48.7%), Nigeria (45.7%)[23,24]. However, one study from south India and turkey found the prevalence of *E. coli* to be higher (68.8% and 82.3%, respectively) than the present study[25–26]. M. Haghi-Ashteiani *et al*. stated *Klebsiella* spp. as the second most frequently isolated organism[27]. This finding is consistent with the present study where *Klebsiella* spp. was also found to be the second most prevalent organism causing UTI (18.9%).

The resistance observed to ciprofloxacin in this study was higher in *E*. coli, *Klebsiella* spp., and *A. baumannii* with susceptibility rates of only 21.0%, 43.0% and 55.0%, respectively. This is similar to other studies in which uropathogens showed higher fluoroquinolones resistance[7,12]. Dash *et al*. reported a much lower resistance rate of *E. coli* against ciprofloxacin compared to our study[25].

The susceptibility of Gram-negative uropathogen to carbapenems are almost similar in our study, with meropenem showing the highest sensitivity. However, a study from Uttar Pradesh reported a much higher sensitivity of uropathogens to carbapenems[19]. In the present study, The overall 58.0% ESBL positive organisms were observed including 61% *E. coli* and 72% *Klebsiella spp*. This is almost similar to a study by Eshwarappa M *et al*. from India who found ESBL positivity to be 52.2%[7]. In contrast to this study, the prevalence of ESBL was found to be 45.1% in Delhi as reported by Kaur N *et al*.[28].

Among the *S. aureus* isolates in our study, highest sensitivity was seen to vancomycin (100%), linezolid (100%) and followed by nitrofurantoin (92%). However, in the study in Nigeria, the sensitivity of *S. aureus* to nitrofurantoin was lower (60%)[29]. According to study by Patel SS *et al*., the average six-year prevalence of MRSA was found to be 37.0% which is similar to our study (35.0%)[30]. In a five-year study among urine isolates by Looney AT *et al*. , MRSA was found to be in 28.0% isolates of *S. aureus*[32]. There has been difference in the resistance of vancomycin in *Enterococcus* spp. with range of 7.6%-10.3% in a study of 2019-2020 by Smout E *et al*.[31]. In this study also, the prevalence is similar for vancomycin-resistant enterocci which is 6.0%. There has been constant increase in the level of vancomycin-resistant enterocci cases since 2000[32].

Thus as per the observations in the present study we conclude that the following antimicrobials may be good options for starting empirical therapy for different commonly isolated organisms at present in our institute. Aminoglycosides, meropenem, piperacillin-tazobactam and nitrofurantoin for *E. coli*; piperacillin-tazobactam, aminoglycosides, meropenem and norfloxacin for *Klebsiella* spp.; piperacillin-tazobactam, fluroquinolones and meropenem for *P. aeruginosa*; tetracycline, doxycycline, minocycline and piperacillin-tazobactam for *A. baumannii*; aminoglycosides, nitrofurantoin, tetracycline, doxycycline, minocycline, glycopeptides and linezolid for *S. aureus*; nitrofurantoin, glycopeptides and linezolid for *Enterococcus* spp. All the Gram-negative isolates were sensitive to colistin but it should be used with caution and only if no other alternative is available. ESBL producing *E. coli* and *Klebsiella* spp. isolates seem to be increasing over the years and were found in over 60.0% of the isolates so empirical therapy with Beta lactam antibiotics might not advisable.

5. Conclusions

There have been very few studies from this region of the country depicting the susceptibility patterns of the most common uropathogens over the years. Empirical therapy is largely guided by information on the profile and susceptibility pattern of the organisms prevalent in the area. This study provides valuable insight regarding the antimicrobial resistance among uropathogens in our hospital. It highlights that antimicrobial susceptibility test data may vary in different areas and over different time periods, thus impressing a need for continuous surveillance. Judicious use of antimicrobial agents is pivotal to the control of antimicrobial resistance. This locally generated antimicrobial susceptibility test data will contribute significantly to guiding empirical treatment choices, tailoring the antibiotic stewardship programs, and also serving as an alert against increasing multidrug-resistant strains.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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This study received no extramural funding.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

William A prepared and revised the manuscript. Rawat D supervised, checked corrections and revised the manuscript. Satija S supervised and revised the manuscript. Kaur R supervised and checked corrections in the manuscript.

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