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PREVALENCE OF BACTERIOLOGICAL AND ANTIBIOTIC RESISTANCE PROFILE AMONG URINARY TRACT INFECTION PATIENTS: A STUDY FROM A MULTISPECIALITIES HOSPITAL IN ANDHRA PRADESH, INDIA

S. Janardhana Reddy[1], R. Alekhya[1], Sripriya A.[2], P. Vijaya[2], V. Anuradha[3] and Meenakshi Kante[1]*

[1]Department of Microbiology, Vignan P.G College, Guntur.
[2]Department of Microbiology, Lalitha Super Specialities Hospital, Guntur.
[3]Department of Chemistry, Vignan P.G College, Guntur.

***Corresponding Author: Dr. Meenakshi Kante**
Department of Microbiology, Vignan P.G College, Guntur.

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ABSTRACT

Introduction: Any portion of the urinary system, including the kidneys, ureters, bladder, and urethra, can get infected. We refer to it as an infection of the urinary tract. The most prevalent uropathogen, *Escherichia coli*, is responsible for about 80% of UTIs. The prevalence of medication resistance in urinary tract infections has been rising globally. The improper and careless use of antibiotics causes bacteria to develop antibiotic resistance. The current study's objective is to identify frequent UTI-causing bacteria and the patterns of antibiotic resistance they exhibit. **Methods:** Urinary pathogens were identified by culture and antibiogram. **Results**: During the study period from December 2023 to February 2024, a total of 200 patients were enrolled with the largest proportion coming from Gynaecology (55%) followed by Medicine (26%) and Urology (19%). Among the 145 cases that tested positive for cultures, E. coli emerged as the most prevalent uropathogen, particularly among individuals aged 41 to 60. In patients younger than 20 years, no uropathogens were detected. Regarding gender, E. coli was the most common in both male and female patients. Analysis of antibiotic resistance showed significant resistance of E. coli to ampicillin, with varying levels of resistance among Gram-negative and Gram-positive isolates. **Conclusion:** This research highlights the growing issue of antimicrobial resistance in uropathogens and the necessity for tailored antibiotic strategies based on specific regions. Empirical management of urinary tract infections should be informed by regional resistance trends to enhance patient outcomes and mitigate the spread of multidrug-resistant infections.

KEYWORDS: Urinary Tract Infections**,** Uropathogenic *Escherichia coli*, culture, Drug Resistance, Bacterial Drug Therapy.

INTRODUCTION

Urinary tract infections preface

Any portion of the urinary system, including the kidneys, ureters, bladder, and urethra, can get infected. We refer to it as an infection of the urinary tract. CA-UTI stands for community-acquired urinary tract infection. Nosocomial urinary tract infections (N-UTIs) are urinary tract infections that happen within 48 hours of hospitalization. Most often, urinary tract infections (UTIs) are caused by bacteria that penetrate the vaginal opening after colonizing the perianal area. More than 10% of women in the US get a UTI annually, compared to just 3% of males. This indicates that women are more likely than men to get a UTI. UTI risk factors include immunosuppression, neurological illness, age, gender, sexual history, and pregnancy.[1,2]

A UTI can be categorized as asymptomatic or asymptomatic, complicated or uncomplicated, acute or chronic, depending on which parts of the tract are affected.[3] The most prevalent uropathogen, *Escherichia coli*, is responsible for about 80% of UTIs. Along with the fungus Candida albicans, other bacteria that cause UTIs include gram-negative bacteria like *Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter*, and gram-positive cocci like *Staphylococcus aureus.*[4]

Culture media for isolating uropathogens: A urine culture is necessary in order to determine which bacteria cause UTIs. A wide range of conventional media, including blood agar (BA), Mac conkey agar (MA), nutritional agar (NA), and cysteine lactose electrolyte deficient (CLED) agar, is used in most labs. HiCrome

UTI Agar is a sophisticated and potent medium designed especially to help identify urine bacteria.[5]

Antimicrobial therapy foreword: The treatment of UTIs has greatly benefited from the advent of antibiotic therapy. The antimicrobial drugs used to treat UTIs include third-generation cephalosporins, DNA gyrase inhibitors, aminoglycosides, which are inhibitors of protein synthesis, and cell wall inhibitors like penicillin.[6]

Scientific problem addressed in this study

For the past few decades, the prevalence of medication resistance in urinary tract infections has been rising globally. Wide-spectrum antibiotics are frequently used to treat UTIs, and treatment is initiated based on anecdotal evidence rather than sensitivity and culture testing. The improper and careless use of antibiotics causes bacteria to develop antibiotic resistance.[7]

The antimicrobial sensitivity patterns and distribution of uropathogens can vary from one location to another. According to the background mentioned above, appropriate therapy is necessary to lessen or prevent resistant strains. Understanding UTI-causing organisms and their antibiogram is essential to ensuring proper treatment.

Thus, we have designed the study to analyze data from hospital settings regarding the organisms that cause UTIs and their patterns of antibiotic resistance. The current study's objective is to identify frequent UTI-causing bacteria and the patterns of antibiotic resistance they exhibit.

METHODS

This is a prospective study of Vignan degree and P.G College and this project was conducted at Lalitha super specialities hospital, Guntur, Andhra Pradesh, India from December 2023 to February 2024. Written informed consent was obtained from all the patients/guardian and next responsible attendant as applicable.

Ethical statement

For this study, ethical approval was obtained from the Lalitha Super Specialities Hospital Ethics committee (LSSH-EC Regd no. ECR/437/INST/AP/2013/RR-19).

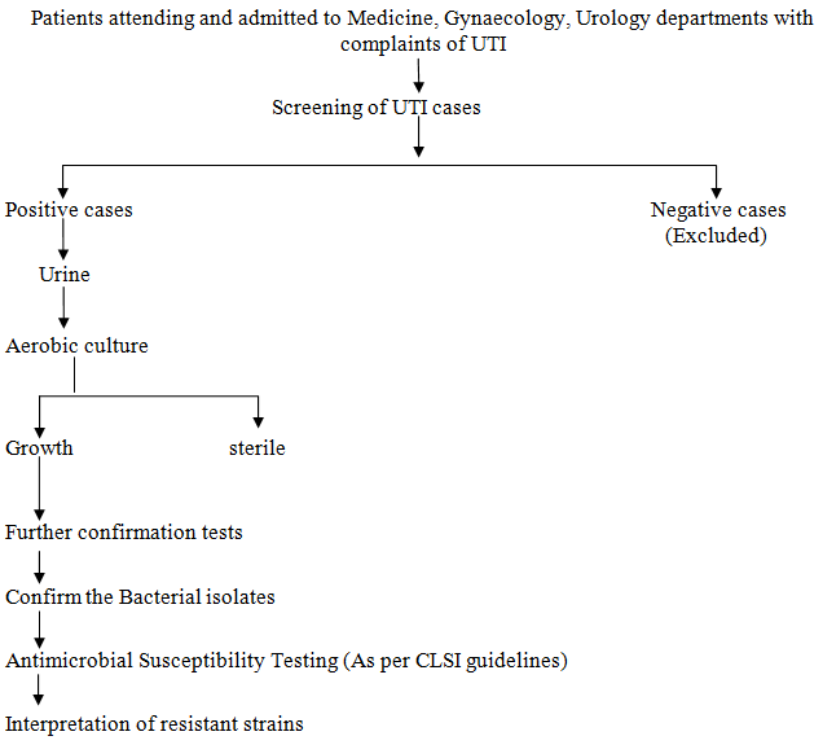
Sample size: 200

Type of study

Prospective cross sectional study.

The movement of the sample from collection to processing is shown in the following flow chart -

Flow chart depicting Study Design



Collection and Processing of Samples

Patients admitted to the departments of medicine, gynecology, and urology provided 270 urine samples in total. This study comprised 200 clean-catch midstream or catheter-derived samples for aerobic culture and antibiotic susceptibility testing based on inclusion/exclusion criteria, informed consent, and sample adequacy. Urine was taken midstream in accordance with Koneman's instructions.[8] Males were told to perform basic urethral meatus cleaning, while females were instructed on perineal washing and labia separation. After the region was cleaned, samples from indwelling catheters were aspirated aseptically from the connector tubing using a 28G needle and syringe. Samples were processed in two hours after being transferred right away.

Quality Control and Media Preparation

The manufacturer's instructions for making chromogenic HiCrome UTI agar (HiMedia) were followed: 55.44g/L, autoclaved at 121°C for 15 minutes, poured at 45–50°C, pH 7–7.4, and kept at 2–8°C for up to two weeks. S. aureus ATCC 25923, E. coli ATCC 25922, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 27853, E. faecalis ATCC 29212, and *P. mirabilis* ATCC 12453 were the ATCC reference strains used to quality-control each batch. Similar preparations and validations were made for other media, including Blood agar, Nutrient agar, MacConkey, and CLED agar.[9]

Initial Identification and Culture

Following quadrant streaking, uncentrifuged urine samples were inoculated on all medium using a calibrated 4 mm loop (0.01 mL). For twenty-four hours, plates were incubated at 37°C. The colony count was computed as follows: cfu/mL = colony count times 100. The Kass criteria[10] identified significant bacteriuria as $\geq 10^5$ cfu/mL. On the basis of colony color and morphology (e.g., *E. coli* is pink, *Klebsiella* is metallic blue, *Enterococcus* is dry blue, and *Pseudomonas* is green), chromogenic agar was utilized for initial identification.[11]

Identification by Biochemical reactions

Standard biochemical tests and Gram staining were used to further validate the isolates[12–15]:

For Gram-positive cocci (QC: *S. aureus, S. pyogenes*), catalase and coagulase are essential

- The Indole test for *E. coli* (Ehrlich's technique)
- TDA (ferric chloride reagent) for the detection of *Proteus*
- Use of citrate in Simmons' medium (QC: *E. aerogenes, E. coli*) Christensen's medium (QC: *Proteus, E. coli)* is used to urease. Using Kligler Iron Agar (KIA) to produce H_2S and ferment carbohydrates
- The Bile Esculin test for *Enterococcus* species (QC: *S. viridans, E. faecalis*)

According to CLSI 2020 recommendations, Mueller-Hinton Agar (HiMedia) was subjected to the Kirby-Bauer disk diffusion method for antibiotic susceptibility testing.[16] The following antibiotics were evaluated for Gram-negative isolates: ampicillin, gentamicin, amikacin, ceftriaxone, ceftazidime, meropenem, ciprofloxacin, nitrofurantoin, and colistin; linezolid, vancomycin, amoxicillin/clavulanic acid, and others were used for Gram-positive bacteria. Using broth microdilution, the minimum inhibitory concentrations (MICs) for vancomycin (*S. aureus, Enterococcus*) and colistin (Gram-negatives) were established. Every MHA plate was made to the same depth (4 mm) and pH range (7.2–7.4).

With ATCC strains (*E. coli* 25922, *S. aureus* 25923), quality control was carried out.[17]

Quality Control

The effectiveness of the autoclave was assessed utilizing tape indicators, daily temperature recording, equipment calibration, and validation of all reagents as part of quality assurance procedures. During the pre-analytical and post-analytical stages, both positive and negative control strains were employed.

All data is entered into Microsoft Excel.

Data analysis: Mean and standard deviation (SD) will be used to describe all continuous variables, while mean will be used to summarize other variables. Percentages will be used to represent categorical variables. The SPSS Windows 20 versions will be used to do the whole data analysis.

RESULTS

The study period from December 2023 to February 2024, a total of 200 patients were screened, of which based on inclusion and exclusion criteria 200 patients were included in the study for sample collection and further analysis. Patients attending and admitted to various clinical departments as detailed in Table 1. Of the 200 cases highest cases was from department of gynaecology 110 (55%) in that positive cases 88 (60.8%) followed by Medicine 52 (26%) in that positive cases 30 (20.6%) and urology 38 (19%) in that positive cases 27 (18.6%) in decreasing order.

Figure 1: Total number of organisms isolated in Urine culture.

Age wise distribution of Uropathogens isolated in urine culture depicted in Table 2. Of the 200 cases, no organisms were isolated in <20 years age group. In the age group 21-40, highest uropathogen isolated in culture was *E.coli* 10 (5%), lowest *Citrobacter*, *Enterococcus* & *Proteus* was equal distribution of 1 (0.5%). The highest uropathogen was *E.coli* 26 (13%) isolated in the age group (41-60) and lowest *Citrobacter & Coag-Neg-Staph* was equal distribution of 1 (0.5%). In the age group 61-80, highest uropathogen isolated in culture was *E.coli* 6 (3%) and lowest *Enterococcus* & *Enterobacter* was equal distribution of 1 (0.5%). The highest uropathogen isolated *Klebsiella* 10 (5%) in the age group above 80 and lowest *Staphylococcus aureus, Enterococcus & Proteus* was equal distribution of 1 (0.5%).

Gender wise distribution of uropathogens isolated in urine culture depicted in table 3. Of the 200 cases, 145 were culture positive and no growth was observed in 55 cases. Highest uropathogen reported as *E.coli* 29 (14.5%) in male and 18 (9%) and lowest was *Citrobacter* 2 ((1%).

Among Female group highest uropathogen reported as *E.coli* 18 (9%) and lowest was *Enterococcus* 1 (0.5%).

Different antibiotics used for this study. 39 *E.coli* isolates have shown highest resistant to ampicillin when compared to other drugs. Different resistance pattern were observed among gram negative bacilli organisms are represented in Table 4.

Different resistance pattern were observed among gram positive cocci (*Enterococcus, Staphylococcus aureus and coagulase negative staphylococci)* are represented in Table 5.

Table 1: Department wise distribution of various samples.

Department	Total cases	Positive cases
Gynaecology	110 (55%)	88 (60.8%)
Medicine	52 (26%)	30 (20.6%)
Urology	38 (19%)	27 (18.6%)
	200	145

Table 2: Age wise distribution of Uropathogens isolated in Urine culture.

ORGANISM	<20 Years	20-40 Years	41-60 Years	61-80 Years	>80 Years
E.coli	Nil	10(5%)	26(13%)	6(3%)	5(2.5%)
Klebsiella	Nil	2(1%)	10(5%)	2(1%)	10(5%)
Staphylococcus	Nil	Nill	2(1%)	3(1.5%)	1(0.5%)
Acinetobacter	Nil	3(1.5%)	7(3.5%)	Nill	Nill
Citrobacter	Nil	1(0.5%)	1(0.5%)	Nill	Nill
Enterococcus	Nil	1(0.5%)	2(1%)	1(0.5%)	1(0.5%)
Enterobacter	Nil	Nill	7(3.5%)	1(0.5%)	Nill
Coag-Neg-Staph	Nil	2(1%)	1(0.5%)	3(1.5%)	2(1%)
Proteus	Nil	1(0.5%)	2(1%)	2(1%)	1(0.5%)
Pseudomonas	Nil	3(1.5%)	6(3%)	Nill	Nill
Candida	Nil	4(2%)	11(10.5%)	3(1.5%)	2(1%)
Sterile	Nil	3(1.5%)	44(22%)	3(1.5%)	5(2.5%)

Table 3: Gender wise distribution of uropathogens isolated in Urine culture.

ORGANISM	MALE	FEMALE
E.coli	29 (14.5%)	18 (9%)
Klebsiella	17 (8.5%)	7 (3.5%)
Staphylococcus	4 (2%)	2 (1%)
Acinetobacter	4 (2%)	6 (3%)
Citrobacter	2 (1%)	Nil
Enterococcus	4 (2%)	1 (0.5%)
Enterobacter	4 (2%)	4 (2%)
Coag-Neg-Staph	5 (2.5%)	3 (2.5%)
Proteus	4 (2%)	2 (1%)
Pseudomonas	5 (2.5%)	4 (2%)
Candida	12 (6%)	8 (4%)
Sterile	30 (15%)	25 (12.5%)

Table 4: Antibiotic Resistance Pattern Of Gram Negative Bacilli.

ANTIBIOTICS	ORGANISMS						
	E.COLI	***KLEBSIELLA***	***ACINETOBACTER***	***CITROBACTER***	***ENTEROBACTER***	***PROTEUS***	***PSEUDOMONAS***
PIPERACILLIN+TAZOBACTUM	11 (10.5%)	2 (1%)	1 (0.5%)	0	0	1(0.5%)	0
CEFOXITIN	23 (11.5%)	6 (3%)	4 (2%)	1(0.5%)	2(1%)	1(0.5%)	2 (1%)
CEFUROXIME	29 (14.5%)	11 (10.5%)	4 (2%)	2(1%)		1(0.5%)	4 (2%)
CEFEPIME	33 (16.5%)	4 (2%)	5 (2.5%)	1 (0.5%)	1(0.5%)	0	4 (2%)
IMIPENEM	1 (0.5%)	0	1 (0.5%)	0	0	0	1 (0.5%)
MEROPENUM	23 (11.5%)	13 (6.5%)	5 (2.5%)	0	1(0.5%)	1 (0.5%)	6 (3%)
ERTAPENEM	23 (11.5%)	9 (4.5%)	8 (4%)	0	1(0.5%)	0	7 (3.5%)
AMIKACIN	6 (3%)	2(1%)	4 (2%)	0	0	0	1 (0.5%)
TETRACYCLINE	0	0	0	0	2(1%)	0	0
MINOCYCLINE	1 (0.5%)	0	0	0	0	0	0
AZITHROMYCIN	20 (10%)	5 (2.5%)	3 (1.5%)	1 (0.5%)	0	2(1%)	6 (3%)
CIPROFLOXACIN	30 (15%)	10 (5%)	2 (1%)	2 (1%)	0	0	2 (1%)
COTRIMOXAZOLE	18 (9%)	7 (3.5%)	4 (2%)	1 (0.5%	1(0.5%)	2(1%)	5 (2.5%)
CEFOPERAZONE+SULBACTUM	26 (13%)	12 (6.5%)	3 (1.5%)	2 (1%)	2(1%)	0	5 (2.5%)
NITROFURANTOIN	19 (9.5%)	13 (6.5%)	8 (4%)	0	0	2(1%)	2 (1%)
AMPICILLIN	39 (19.5%)	18 (9%)	10 (5%)	2 (1%)	4(2%)	3(1.5%)	8 (4%)
CLINDAMYCIN	0	0	0	0	2(1%)	0	0

Table 5: Antibiotic Resistance Pattern Of Gram Positive Cocci.

ANTIBIOTICS	ORGANISMS		
	Enterococcus	***Staphylococcus***	***Coag-Neg-Staph***
CEFOXITIN	1 (0.5%)	2 (1%)	2 (1%)
CEFUROXIME	0	0	0
CEFEPIME	1 (0.5%)	0	0
IMIPENEM	0	0	0
MEROPENUM	0	0	0
ERTAPENEM	0	0	0
GENTAMYCIN	0	0	0
HIGH LEVEL GENTAMYCIN	5 (2.5%)	0	0
AMIKACIN	0	0	0
TETRACYCLINE	6 (3%)	1 (0.5%)	1 (0.5%)
AZITHROMYCIN	0	0	0
CIPROFLOXACIN	0	0	0
COTRIMAXAZOLE	2 (1%)	1 (0.5%)	0
CEFOPERAZONE+SULBACTUM	1 (0.5%)	0	0
NITROFURANTOIN	0	0	0
AMPICILLIN	7 (3.5%)	4 (2%)	5 (2.5%)
CLINDAMYCIN	6 (3%)	0	0

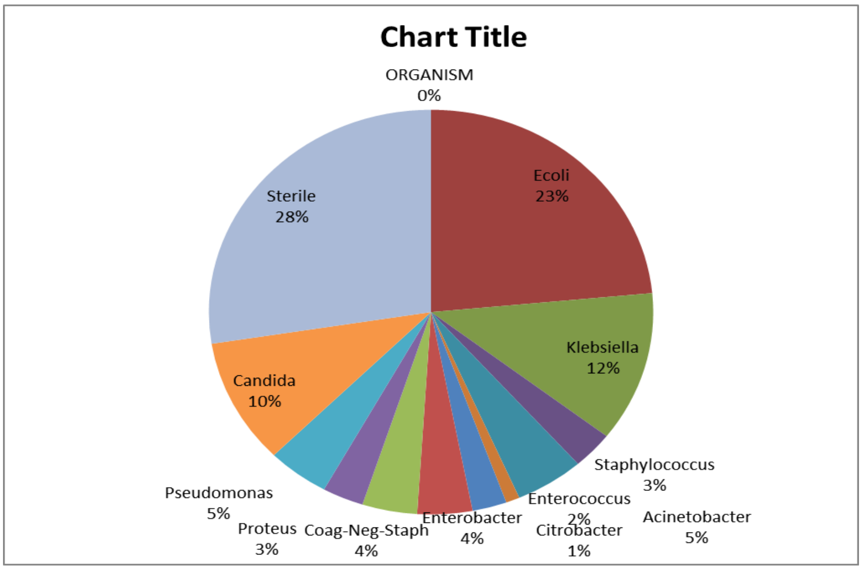


Figure1: Total number of organisms isolated in Urine culture.

Figure 2 : Growth on Hichrome UTI agar Medium.

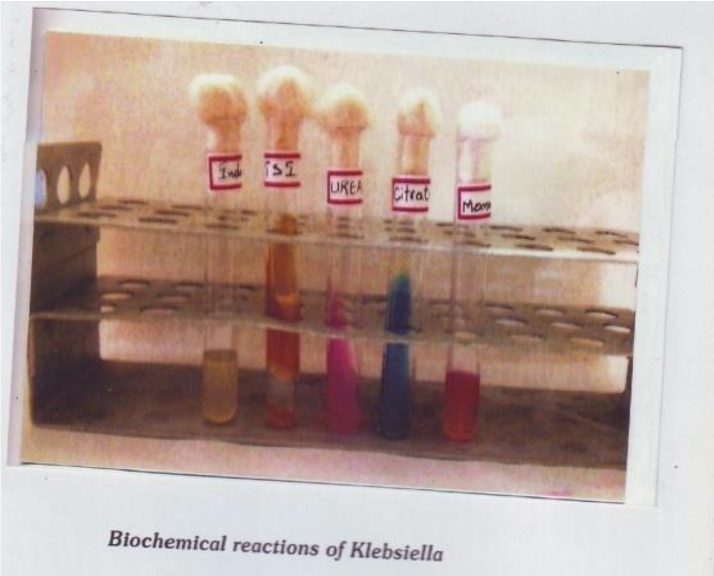


Figure 3: Biochemical reactions of *Klebsiella*.

Figure 4 : Antibiogram of UTI pathogens.

DISCUSSION

This prospective study was carried out at Lalitha Super Specialties Hospital in Guntur, Andhra Pradesh, India, during the period from December 2023 to February 2024. Over the years, the etiology causes, and antimicrobial susceptibility patterns of uropathogens have evolved. According to *Ghadage DP et al*[18], effective management of patients with bacterial urinary tract infections mainly depends on identifying the organisms responsible for the infection. The diagnosis of urinary tract infections highlights the importance of close collaboration between clinicians and microbiologists, as noted by *Moue A in 2015*.[19] Given this context, it is essential to conduct studies that identify uropathogens and their resistance to antibiotics. Consequently, we carried out the present study to detect a variety of bacterial isolates and the antibiotic resistance patterns among patients with urinary tract infections. We gathered urine samples for this research from various departments, including Urology, Gynecology, and Medicine. The study found the highest number of cases originating from the Gynecology department, with 110 cases (58%), which aligns with the findings reported by *Kumar et al.*[20] in 2023.

Out of the 200 patients in the current study, *E. coli* was the most prevalent uropathogen (23.5%), followed by *Klebsiella* (12%), *Candida* (10%), and other bacteria. Moreover, the most often found pathogen in nosocomial catheter-related and noncatheter-related infections was E. coli (23.5%). These results suggest that the main cause of UTIs is *E. coli*.[21]

Among gram-negative uropathogens, *Escherichia coli* is the most commonly encountered pathogen responsible for urinary tract infections (UTIs), which aligns with findings from previous research by *Arora et al. (2016)*[22], *Stefaniuk et al. (2016)*[23], *Ghadage et al. (2016)*[18], *Odoki et al. (2019)*[1], *Chen et al. (2020)*[24], *Daoud et al. (2022)*[25], *Huang et al. (2022)*[26], *and Jagadeesan et al.* (2022).[27]

The highest incidence of *E. coli* (13%) was observed in the 41-60 age group, which encompasses individuals aged 20 to 80. *E. coli* was identified as being more common in males (29.5%) compared to females (18.9%). *Klebsiella pneumoniae* is the second most prevalent uropathogen associated with UTIs, representing 24 cases (12%). This observation supports the findings of *Ghadage et al. (2016)* while contradicting those of *Tambekar DH et al.*[21] Citrobacter was determined to be the least common uropathogen in our sample, comprising only 1%. *Klebsiella pneumoniae* was recorded at 10 cases (5%), with the highest occurrences in the age of 41-60 and above 80, showing an equal distribution. Additionally, *Klebsiella* pneumonia was found to be more common in males (17.5%) than females (7.5%).

The strain *Staphylococcus aureus* was identified in three (1.5%) of the gram-positive uropathogens. Males were found to have four (2%) instances compared to two (1%) in females. According to the findings of this study, gram-negative pathogens are more prevalent in urinary tract infections (UTIs) than gram-positive ones. The rise in gram-negative bacteria from the Enterobacteriaceae family causing UTIs can be linked to several factors, such as their ability to attach to the uroepithelium due to colonization of the urogenital mucosa through adhesins, pili, fimbriae, and the P-1 blood group phenotypic receptor. UTIs are recognized as among the most frequent infections caused by a variety of gram-positive and gram-negative bacteria.[28]

To effectively treat patients experiencing a UTI, it is essential to correctly identify the pathogenic organism. Failing to do so may prolong the illness and lead to complications, as well as foster negative effects of antibiotic resistance due to the improper use of antibiotics. Furthermore, diagnosing a UTI relies on both the presence of clinical signs and symptoms, along with a positive urine culture; however, in many healthcare environments, this diagnosis is made and treatment is initiated without conducting a culture or assessing antimicrobial susceptibility. It is vital to keep current knowledge of the organisms responsible for urinary tract infections and their antibiotic resistance patterns to ensure appropriate treatment.[29]

Antibiotic resistance among gram-negative bacilli was highest for ampicillin, with minimal or no resistance observed for minocycline. *E. coli* exhibited the highest resistance to ampicillin at 39 cases (19.5%), followed by cefepime at 33 cases (16.5%), ciprofloxacin at 30 cases (15%), cefuroxime at 29 cases (14.5%), and cefoperazone plus sulbactam at 26 cases (13%). The resistance for cefoxitin, meropenem, and ertapenem was 23 cases (11.5%), while azithromycin had 20 cases (10%). Nitrogurantoin showed 19 cases (9.5%), cotrimoxazole had 18 cases (9%), and piperacillin plus tazobactam demonstrated 11 cases (10.5%). Amikacin showed 6 cases (3%), minocycline and imipenem had 1 case (0.5%), and there was no resistance to clindamycin or tetracycline, listed in decreasing order. *E. coli* is the most prevalent pathogen responsible for UTIs and has displayed resistance to multiple drugs included in this study compared to other studies, such as Raval R, 2015.[30] In this study, *Klebsiella* displayed resistance at 18 cases (9%), *Acinetobacter* at 10 cases (5%), *Pseudomonas* at 8 cases (4%), *Enterobacter* at 4 cases (2%), and Proteus at 3 cases (1.5%) with the highest resistance to ampicillin. However, Citrobacter showed an equal resistance pattern of 2 cases (1%) to ampicillin, ciprofloxacin, cefuroxime, and cefoperazone plus sulbactam. With the exception of *Enterobacter*, tetracycline and clindamycin demonstrated no resistance among all gram-negative bacilli, and *E. coli* exhibited the least resistance to minocycline at 1 case (0.5%) with no observed resistance (0%) in other gram-negative bacilli, marking these as critical findings. In this study, gram-positive cocci demonstrated lower overall resistance to antibiotics compared to gram-negative bacilli. Among the gram-positive cocci, *Enterococci* showed the highest resistance to ampicillin with 7 cases (3.5%), followed by coagulase-negative staphylococci at 5 cases (2.5%), and *Staphylococcus aureus* at 4 cases (2%) in descending order.

CONCLUSION

The increasing resistance of bacterial uropathogens is emerging as a public health concern in India. Numerous cities and towns in India lack adequate microbiological laboratories, resulting in limited microbiological evaluations and a rise in the empirical use of antibiotics. Typically, urine samples are only sent for microbiological analysis after treatment failures or instances of recurring or relapsing infections. Our results highlight the importance of understanding local antibiotic resistance patterns, which can then inform the development of antibiotic policies at both hospital and regional levels. Inappropriate practices by healthcare providers, such as inaccurate prescriptions or excessive antibiotic use, are viewed as significant factors contributing to the development and spread of bacterial resistance. Physicians often adhere to broad guidelines when managing patients with urinary tract infections (UTIs). Consequently, the local trends in epidemiology and antimicrobial susceptibility rates regarding common bacteria are commonly overlooked. In this research, we concentrated on the prevalence, causative agents, and trends in antimicrobial resistance of uropathogens. This study could offer valuable information for making effective antimicrobial choices for UTI treatments. We strongly advocate that any empirical antibiotic choice should take into account local epidemiological data and resistance trends for the prevalent uropathogens instead of relying on a one-size-fits-all guideline. The results could also provide a foundation for creating new policies aimed at curbing the rise of multidrug-resistant uropathogens.

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DECLARATIONS

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Conflict of interest: Nil.

Ethical approval: Obtained.

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