

Prevalence of Uropathogens and their Antibiotic Sensitivity Pattern in a Rural Medical College Hospital

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Abstract:

Background: Urinary tract infections represent a substantial proportion of patients seen in clinical departments at Medical College Hospitals. They result in considerable morbidity and death in individuals exhibiting heightened antibiotic resistance. This often results in the indiscriminate, unscientific administration of antibiotics, leading to the rise of multidrug-resistant bacteria in hospitals.

Aim: The aim of this research is to determine the prevalence and sensitivity patterns of different uropathogens and to recommend empirical antibiotics within the clinical environment of a medical college hospital.

Materials and Methods: A retrospective research was conducted over one year (2022-2023), during which 1008 urine samples taken from clinical departments were analyzed at the Microbiology department at a Tertiary Health Care Centre in Bengaluru, Karnataka, India. Urine samples underwent bacterial culture, revealing substantial bacterial growth by the colony count technique. Traditional techniques were used for the identification of bacterial isolates. Antimicrobial susceptibility testing was conducted on isolates in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations.

Results: Out of 1008 urine samples, 388 samples (38.49%) exhibited considerable proliferation of uropathogens. The predominant uropathogens discovered were *Escherichia coli* (37.03%), followed by *Klebsiella pneumoniae* (14.5%), *Enterococcus* species (10.71%), Methicillin-Resistant Coagulase-Negative Staphylococci (MRCONS) (9.17%), and *Pseudomonas aeruginosa* (6.8%). Multidrug resistance has been shown in many uropathogens, particularly to penicillin's, fluoroquinolones, and cephalosporins.

Conclusion: This research recorded a rise in antibiotic resistance among uropathogens. The empirical selection of antimicrobial agents should be predicated on the antimicrobial sensitivity profiles of the uropathogens endemic to the region. Prudent utilization of antibiotics may assist in mitigating the dissemination of antimicrobial medication resistance amongst the general populace.

Keywords: Urinary tract infections, *Klebsiella pneumoniae*, Staphylococci, *Pseudomonas aeruginosa*, *Escherichia coli*.

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Introduction

Urinary tract infections (UTIs) are a prominent category of illnesses throughout the society, particularly in South Asian nations. A decade-long investigation by the Global Prevalence investigation on Infections in Urology (GPIU) found that urinary tract infections (UTIs) exhibit significant antimicrobial resistance to broad-spectrum antibiotics and underscored the need for effective UTI care, particularly in Asian nations. [1] A research conducted in African nations also shown a correlation between UTI isolates and

elevated bacterial antibiotic resistance. The country of Ethiopia performed a laboratory cross-sectional investigation and identified uropathogens that were sensitive only to Gentamicin and Chloramphenicol. [2] India, being one of the most populated countries globally, is linked to a significant prevalence of UTIs caused by drug-resistant organisms. Numerous population studies conducted throughout various areas of India have shown the isolation of diverse bacteria exhibiting markedly varying antibiotic susceptibility patterns. A research

conducted in Maharashtra identified *E. coli* exhibiting significant resistance to Fluoroquinolones, Amoxicillin, and third-generation Cephalosporins, highlighting the need for prompt surveillance and monitoring. [3]

A study conducted by Jannifer, et al. in Chennai, which contradicted the study in Maharashtra, revealed uropathogens that were sensitive to third generation Cephalosporins. [4] A comparative research of the frequency of antimicrobial resistance in community-acquired UTIs from Northern and Southern states of India indicated the separation of several bacteria as the principal pathogens, each linked with distinct patterns of drug resistance. The research authors underscored the need of establishing regional monitoring systems for the effective management and treatment of UTIs. [5]

Recently, there has been a rise in the number of patients presenting with UTI-related problems in outpatient departments. Research indicates a significant rise in drug resistance patterns among uropathogens in outpatient patients, attributed to the improper use of empirical therapy and sporadic overtreatment of these illnesses.

A research conducted in Telangana, a southern Indian state, revealed that urinary tract infections are linked to multidrug-resistant uropathogens and co-morbidities including diabetes, hypertension, and chronic kidney disease. [6]

The changing antibiotic paradigms and the need to use empirical antibiotics against resistant bacteria compelled us to examine the frequency and susceptibility patterns of uropathogens at our tertiary care center.

Materials and Methods

Study design and setting: A retrospective investigation was performed at the Department of Microbiology at a Tertiary Health Care Centre in Bengaluru.

The research assessed urine samples from both outpatient and inpatient departments for bacterial proliferation and antibiotic susceptibility testing for a duration of one year, from November 2022 to December 2023. A total of 1008 urine samples were assessed for the identification and assessment of antibiotic susceptibility of the causal microorganisms.

A urinary tract infection is characterized by the presence of $\geq 10^5$ colony-forming units (CFUs)/mL of bacteria in a mid-stream urine sample, $\geq 10^3$ CFU/mL of a single potential pathogen, two or three organism types with predominant growth of one organism type and $< 10^4$ CFU/mL of other organism types, or $\geq 10^2$ CFU/mL of any number of organism types in suprapubic aspirates and

cystoscopy specimens. A quantitative testing approach is necessary to distinguish real bacteriuria from bacterial contamination resulting from improper urine sample collecting techniques. Viable bacterial colonies are quantitatively assessed per milliliter of urine using the colony count method.

Collection and processing of urine samples

To accurately evaluate genuine bacteriuria, a mid-stream urine sample is obtained in a sterile disposable universal container and inoculated onto Blood agar and MacConkey agar (HiMedia) using the semi-quantitative plating method using a calibrated loop approach (0.001 ml). Culture plates were incubated for 18 hours in ambient air at a temperature of 35–37°C. The colony count technique indicated either substantial or non-significant growth in urine culture. Bacterial colonies were seen on the culture plates by the examination of colony shape and Gram staining. Isolated colonies were discovered by normal laboratory techniques [7,8].

Antimicrobial susceptibility testing was conducted on Mueller Hinton agar using the Kirby Bauer disc diffusion technique, as per the instructions of the Clinical Laboratory Standards Institute (CLSI) [9]. The following antibiotics were evaluated for Gram-negative bacilli (GNB): Amoxyclav (20/10 µg), Amikacin (30 µg), Trimethoprim/Sulphamethoxazole (Cotrimoxazole) (1.25/23.75 µg), Norfloxacin (10 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Gentamicin (10 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Piperacillin/Tazobactam (100/10 µg), Cefoperazone/Sulbactam (75/10 µg), Cefepime (30 µg), Cefixime (5 µg), Doxycycline (30 µg). The antibiotics evaluated for Gram-positive cocci (GPC) included High-Level Gentamicin (120 µg), Cefoxitin (30 µg), Linezolid (30 µg), Vancomycin (30 µg), Norfloxacin (5 µg), Nitrofurantoin (300 µg), and Amoxyclav (20/10 µg). Dehydrated medium and antibiotic discs were obtained from Himedia, India. Organisms exhibiting resistance to any Carbapenem medicines, including Meropenem (10µg) and Imipenem (10µg), with susceptibility zones of ≤ 23 mm were classified as Carbapenem resistant.

Results

During the study period, 1008 urine samples were analysed. 388 urine samples yielded significant growth of pathogen. Table 1 & Bar graph 1. Outlines the demographic profile of patients with UTI. The patients were between the ages of 0 & >44yrs. UTIs were reported in 118(30.41%) males and 270(69.58%) females. Females of the reproductive age group (between 21 to >44yrs) constituted 44.12% of the total population with UTI. The distribution of uropathogens across age

groups revealed the highest prevalence in patients older than 40 years. In this group, E. coli was the most frequently isolated pathogen (19.23%), followed by Enterococcus spp. (11.53%) and Klebsiella pneumoniae (11.23%). The 21-40 years age group also demonstrated significant isolation of E. coli (12.42%) followed by Enterococcus spp. (5.32%), Methicillin resistant Coagulase negative

staphylococci (4.43%), and Methicillin susceptible Coagulase negative staphylococci (4.14%) but with a lower incidence of Klebsiella spp. (1.77%). The lowest prevalence was observed in the 0-20 year's age group, where E. coli accounted for 5.32% of isolates, with minimal representation of other pathogens.

Table 1: Age wise distribution of uropathogens

Age	Enterobacter spp	MIRSA	MSSA	MIRCONS	MSCONS	Enterococci	E. coli	Klebsiella spp	Citrobacter spp	Proteus spp	NFGNB
0-20	1 (0.29%)	1 (0.29%)	0 (0%)	3 (0.88%)	1 (0.29%)	5 (1.47%)	18 (5.32%)	5 (1.47%)	2 (0.59%)	0 (0%)	5 (1.47%)
21-40	2 (0.59%)	3 (0.88%)	4 (1.83%)	15 (4.43%)	14 (4.14%)	18 (5.32%)	42 (12.42%)	6 (1.77%)	2 (0.59%)	0 (0%)	7 (2.07%)
>40	3 (0.88%)	3 (0.88%)	0 (0%)	12 (3.55%)	2 (0.59%)	39 (11.53%)	65 (19.23%)	38 (11.23%)	6 (1.77%)	4 (1.83%)	12 (3.55%)
Total	6 (1.76%)	7 (2.05%)	4 (1.83%)	30 (8.86%)	17 (5.02%)	62 (18.32%)	125 (36.97%)	49 (14.48%)	10 (2.95%)	4 (1.83%)	24 (7.09%)

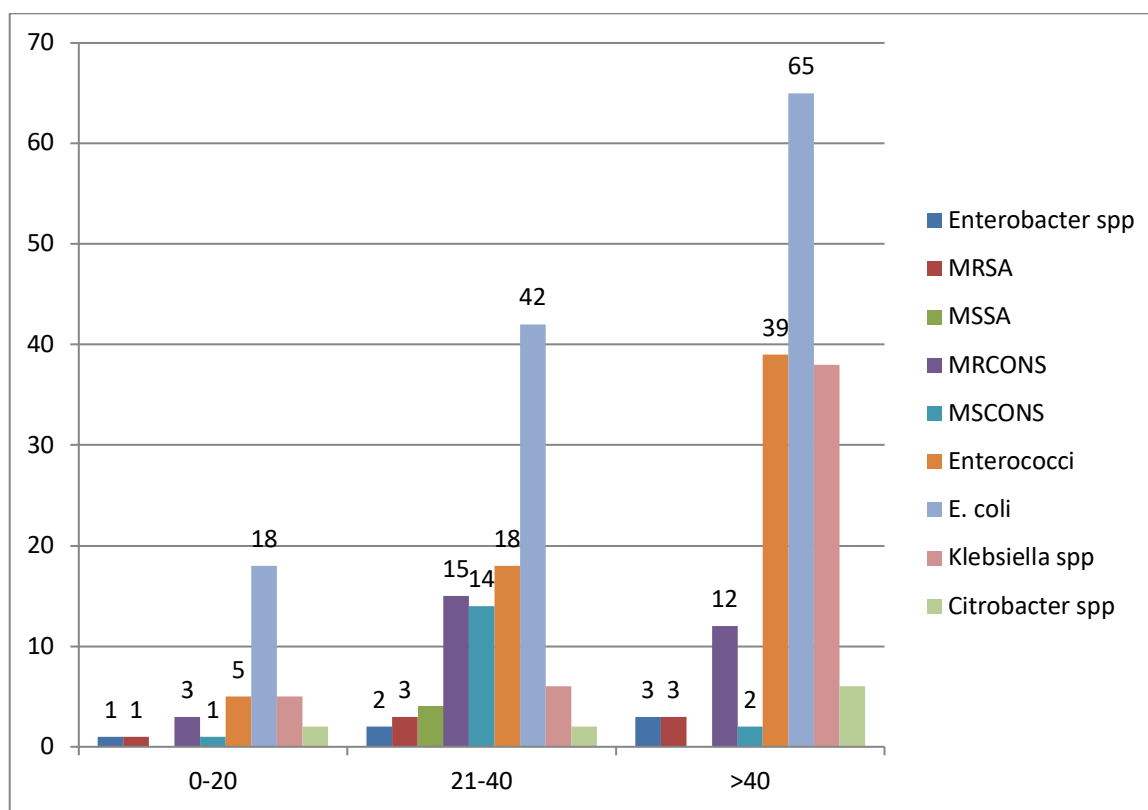


Figure 1: Representation of age wise distribution of uropathogens

The antibiotic resistance patterns of Gram-negative bacteria were assessed using the disc diffusion method following CLSI guidelines.

Escherichia coli, the most common isolate, demonstrated high resistance to several antibiotics, particularly to Fluoroquinolones and Cephalosporins. Resistance to Ciprofloxacin was observed in

62.19% of E. coli isolates, while resistance to Cefotaxime, Cefixime, Cefepime and Cefuroxime was 75%, 80%, 51.40% and 56.97%, respectively. Similarly, Klebsiella pneumoniae exhibited resistance to Cefotaxime (65.95%), Cefixime (72.72%), Cefepime (58.82%) and Cefuroxime (81.81%). The sensitivity pattern of other Gram negative bacilli are shown in table 2 & Figure 2.

Table 2: Antibiotic resistance pattern of GNB

	E.coli	Klebsiella spp	Citrobacter spp	Proteus spp	Enterobacter spp	NFGNB
Amoxyclav	58.92%	53.84%	85.71%	50%	100%	68.75%
Amikacin	16%	41.86%	0	75%	0	61.90%
Co-trimoxazole	57.12%	68.18%	35.71%	100%	50%	78.94%
Ciprofloxacin	62.19%	65%	25%	100%	50%	61.11%
Cefipime	51.40%	58.82%	30.76%	66.66%	50%	63.15%
Cefuroxime	56.97%	81.81%	57.14%	66.66%	100%	82.35%
Cefotaxime	75%	65.95%	57.14%	50%	100%	82.35%
Doxycycline	10.30%	10.86%	10%	100%	50%	31.57%
Gentamicin	29.70%	44.18%	14.28%	75%	50%	72.22%
Imipenem	9.47%	26.82%	15.38%	25%	50%	63.63%
Meropenem	7.56%	27%	20%	25%	50%	58.33%
Piperacillin/tazobactam	19.49%	38.77%	23.07%	25%	50%	50%
Cefixime	80%	72.72%	100%	100%	50%	85.71%
Nitrofurantoin	27.27%	60.86%	76.92%	100%	100%	86.36%
Norfloxacin	52.94%	50%	25%	50%	50%	62.5%
Cefoperazone /sulbactam	21.34%	32.43%	15.38%	66.66%	50%	58.82%

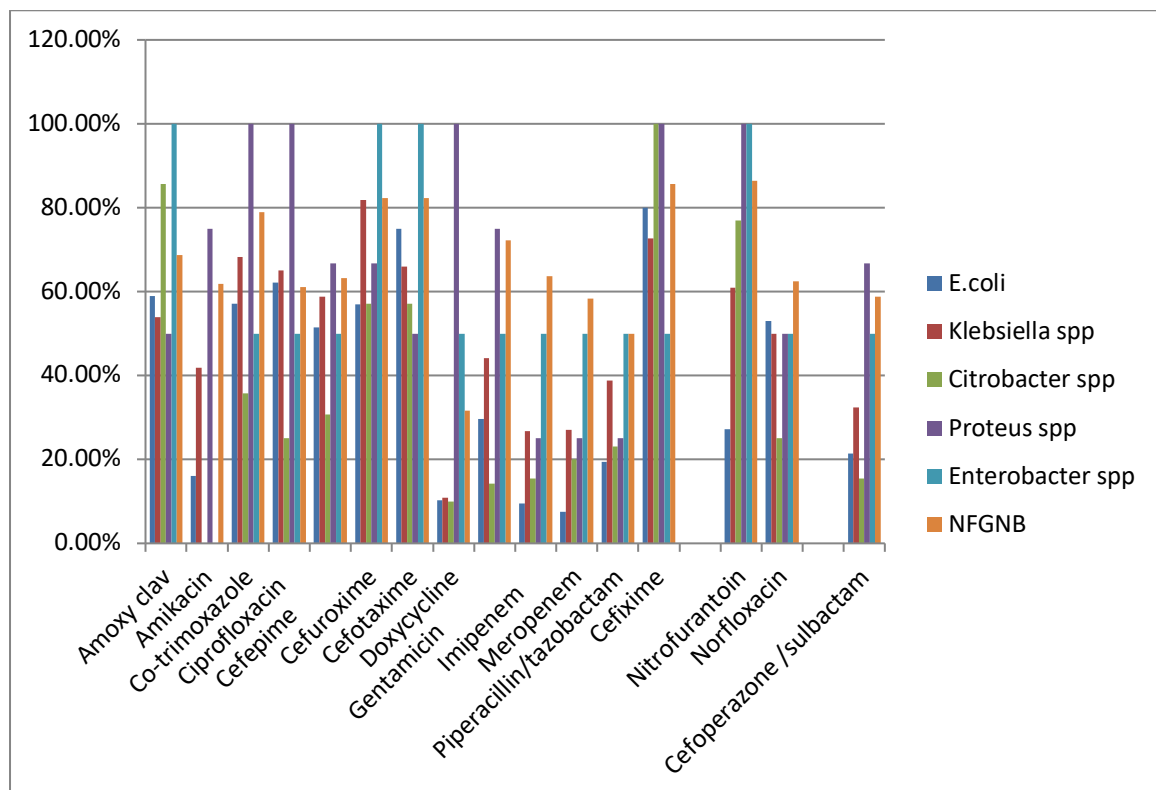


Figure 2: Antibiotic resistance pattern of GNB

The detailed resistance pattern of each gram positive uropathogen isolated from urine specimens, as well as the individual antibiotic susceptibility patterns have been mentioned in the table 3 and depicted in Figure 3.

Table 3: Antibiotic resistance pattern of Gram positive bacteria

	MRSA	MSSA	MSCONS	MRCONS	Enterococci
Amikacin	16.6%	25%	0	16.66%	0
Doxycycline	12.5%	25%	0	0	0
Gentamicin / High level gentamicin	42.85%	25%	15.38%	17.39%	77.19%
Norfloxacin	60%	0	20%	60.71%	90%
Nitrofurantoin	25%	0	13.33%	10.71%	58.33%

Linezolid	0	0	0	0	0
Ciprofloxacin	66.66%	25%	25%	71.42%	75%
Erythromycin	50%	66.66%	43.75%	82.14%	-
Clindamycin	28.57%	33.33%	37.50%	67.85%	-
Penicillin	100%	100%	56.25%	100%	100%
Vancomycin	-	-	-	-	7.01%

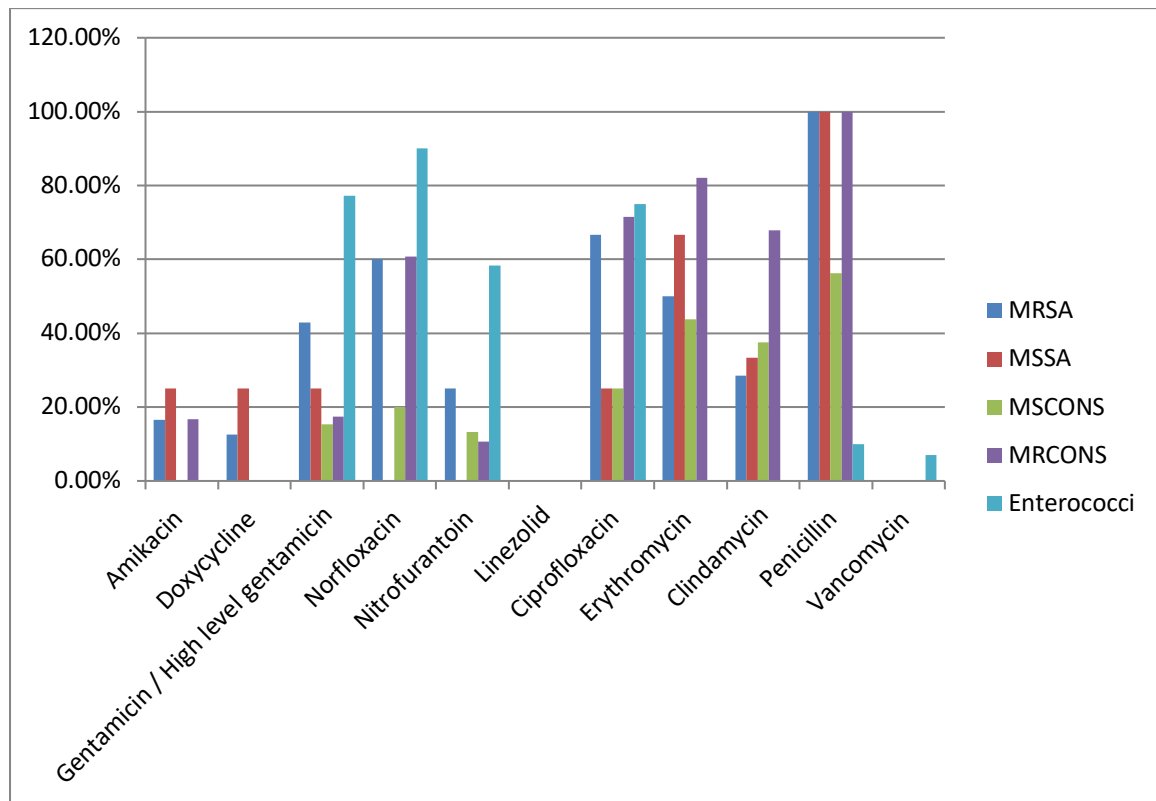


Figure 3: Antibiotic resistance pattern of Gram positive bacteria-all variables

Among Gram positive bacteria, Methicillin-resistant Staphylococcus aureus (MRSA) exhibited 66.66% resistance to Ciprofloxacin, Norfloxacin (60%) and 50% to Erythromycin. All Methicillin susceptible staphylococcal aureus (MSSA) strains showed 100% resistance to Penicillin. No resistance to Norfloxacin and Nitrofurantoin, low resistance to Amikacin, Doxycycline, Gentamicin, Ciprofloxacin (25%). Enterococcus spp. showed significant resistance to Norfloxacin (90%), Ciprof-

loxacin (75%), High-level Gentamicin (77.19%) and Nitrofurantoin (58.33%), but retained susceptibility to Linezolid and Vancomycin, with only 7.01% of Enterococcus spp. exhibiting Vancomycin resistance. Methicillin-susceptible coagulase-negative staphylococci (MSCONS) demonstrated lower resistance rates overall, with no resistance to Amikacin, Doxycycline, and Linezolid. None of the Gram positive bacteria showed resistance to Linezolid.

In our study E.coli shows highest resistance to third generation Cephalosporins (51.4% -75%) followed by Amoxicillin/clavulanic acid (58.9%) and Fluroquinolones (52.9%-62.19%) which is similar to other studies (Smitha sood et al, Farhat et al). Resistance to Nitrofurantoin little high (27.26%) than the findings of study conducted by Smitha sood et

al. But however it is less than the findings of study by Farhat et al (100%).

Comparatively less resistance is shown to Amikacin (53.84%), Nitrofurantoin (27.26%), Gentamicin (29.7%), Cefoperazone / sulbactam (21.34%), & Piperacillin / Tazobactam (19.99%).

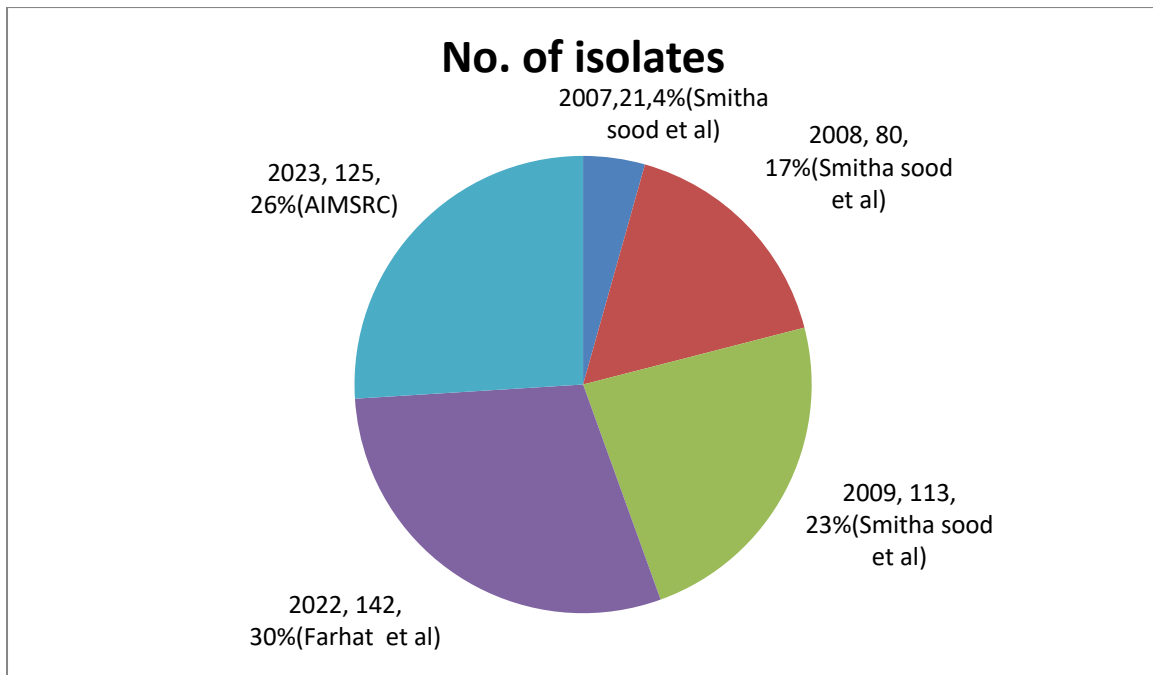


Figure 4: Comparison between different studies of common uropathogen E.coli with antibiotic susceptibility pattern

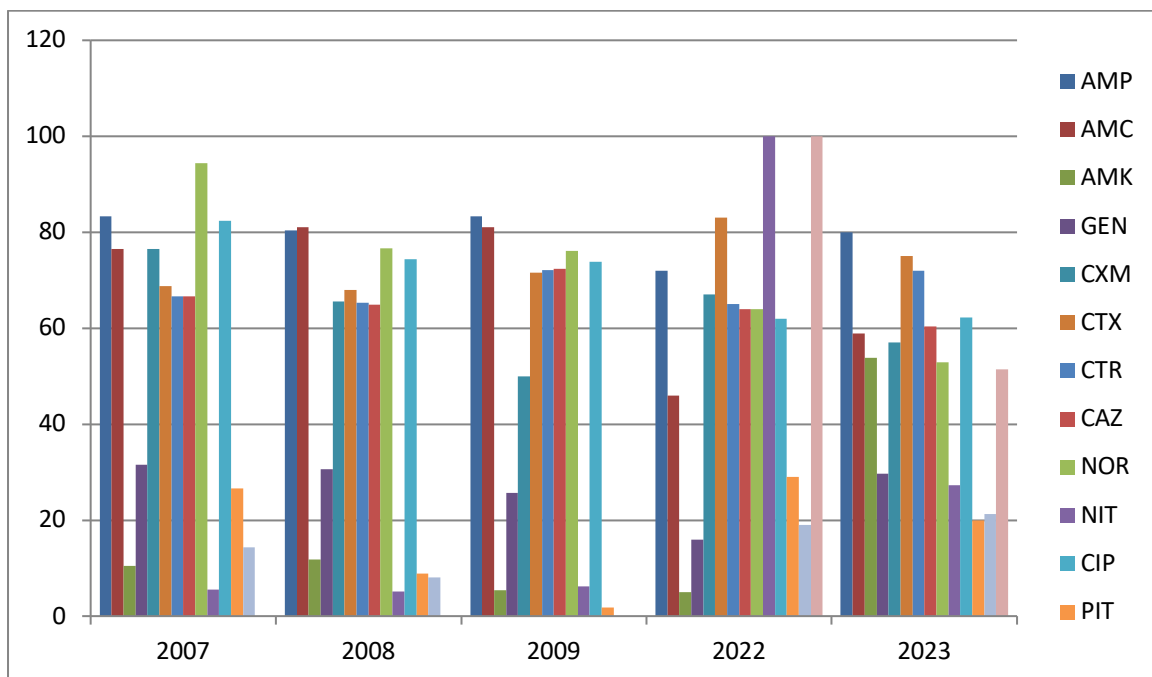


Figure 5: Patterns of ALL antibiotic resistance among organisms isolated 2007 till 2023

Discussion

Urinary tract infections have been described since ancient times with the first documented description in the Ebers Papyrus dated to c. 1550 BC. [10] It is one of the most prevalent diseases in the community and is responsible for 7 million clinic visits annually. [11] There is an increasing trend in multidrug resistance of uropathogens and there are very few new weapons to fight the threat. [12] The possible explanations for antimicrobial resistance include bacterial factors, such as genetic mutations

acquired by the uropathogens, [13] inappropriate use of broad spectrum antibiotics [14] without proper evaluation, lack of evidence based clinical management, 15 incomplete usage of prescribed antimicrobials, and easy access of various broad spectrum antibiotics in the community pharmacy. [16] This is the first study to evaluate the susceptibility patterns of bacterial strains isolated from community acquired UTI 's in our tertiary health care centre. This study gave valuable laboratory data and allows comparison of situation in Bengaluru with other parts of the country.

UTI is one of the most common infections encountered, which affects all age groups, including men, women and children worldwide [17]. The demographic data indicates that women of the reproductive age group (44.12%) formed the main group of adult patients with UTI. UTI's were reported in 69.58% of females and in 30.41% of males. It has been extensively reported that adult women have a higher prevalence of UTI than men, principally owing to anatomic and physical factors. [18] Elderly males (60yrs and >60yrs) had a higher incidence of UTI (32.10%) compared to the elderly females (18.65%). This is probably because, with increasing age, the incidence of UTI increases in men due to prostate enlargement and neurogenic bladder. [19]

The culture positive rate was 38.49% (388/1008) in the present study and a similar culture positive rate has been observed by other studies. [17,20,21]. The antibiotic susceptibility patterns have changed over time but the spectrum of agents causing UTI has remained relatively constant with *E.coli* being the most common isolate [22]. In our study, *E.coli* was the most common isolate (37%). This is similar to studies from other tertiary care centers. [20,21,23] Following *E.coli*, other common uropathogens isolated were *Enterococcus* spp.(18.32%), *Klebsiella* spp.(14.45%), *CONS*(13%) and *NFGNB*(7.09%). Our findings coincide with other studies by Farhat Subhana et al and Smita Sood et al. [23,24] Enterobacteriaceae have several factors responsible for their attachment to the uroepithelium. These gram negative aerobic bacteria colonize the urogenital mucosa with adhesion Pili, fimbriae and P1 blood group phenotype receptor. [19]

Literature review initially revealed that OPD UTI bacterial isolates have been known to be associated with low level bacterial drug resistance. A study from the US from 2001-2010 found only a 2.1% rise in multidrug resistance to Nitrofurantoin, and multidrug-resistant *E.coli* has been found in only 1.4% of cases. Recently, there is a trending increase in bacterial drug resistance in the OPD. [26] A study from Nath, et al. found that there is an increase of antibiotic drug resistance associated with oral formulations, especially Fluoroquinolones and Ampicillin, used in OPD cases. [26]

Our results echo similar findings of Nath, et al. and found an increase in drug resistance, largely to Fluoroquinolones and Cephalosporins. Our results coincide with other studies, as we isolated similar common uropathogens including *E.coli* (37%), *Klebsiella* spp (14.48%) and *Pseudomonas* spp (7.09%). The results were supported by a study conducted by Mohapatra, et al. which revealed that the high prevalence of *E.coli* is associated with an increase in drug resistance. [27]

In our study, isolates of *E. coli* showed higher resistance to Fluoroquinolones and Cephalosporins in coherence with a study by Prasad, et al. which showed increasing resistance to Cephalosporins [28]. In developing countries, an increasing trend of fluoroquinolone resistance in *E.coli* isolates has been noticed. [29] European Association of Urology guidelines have postulated Nitrofurantoin as first line empirical treatment of an uncomplicated UTI. [30] Nitrofurantoin is the drug of choice for treatment, as many of the common uropathogens were found to be highly sensitive to its effects. Resistance pattern of *Klebsiella pneumoniae* and Non-fermenting Gram negative bacilli isolates from our study is similar to the findings of study conducted by Farhat et al, Sood et al and Manjunath et al. [23,24,31]

In contrast to our study, which showed higher resistance to fluoroquinolones and cephalosporins in *Pseudomonas aeruginosa* isolates, a study by Jombo, et al. showed higher sensitivity to Ciprofloxacin (92%) and Cefuroxime (86%), [32] stressing the need of local surveillance of antimicrobial resistance patterns. Antimicrobial susceptibility patterns among Gram positive bacteria such as *Enterococcus* spp. showed higher resistance to Aminoglycosides (77.19%) and Fluoroquinolones (90%) and less resistance to Vancomycin (7.01%) and no resistance to Doxycycline & Linezolid. Similar results were found in a study conducted by Manjunath et al., emphasizing the importance of calculated selection of antibiotics in treating UTI cases to prevent the spread of bacterial drug resistance among drug susceptible organisms. [22]

The current study has certain limitations as it is confined to the people covered by a single tertiary care centre in Bengaluru, India and doesn't necessarily reflect trends in the community. A meta-analysis of antimicrobial susceptibility patterns would provide sufficient information to change and optimize the empirical management of UTIs. This study emphasizes the need for more antimicrobial surveillance at regional, national and international levels. Necessity has aroused the need to promote ideal use of antimicrobials. [33] A decrease in multidrug resistance thereby reduces prevalence of the disease in the community and subsequently improves quality of life.

Conclusion

Escherichia coli was the most prevalent uropathogen across all age groups, with significant resistance to Fluoroquinolones and Cephalosporins. *Klebsiella pneumoniae* and *Enterobacter* spp. showed high rates of resistance to commonly used antibiotics, such as Cefuroxime, Cefixime, Cefotaxime and Amoxycylav. Carbapenem resistance was relatively low among Gram-negative

bacteria, particularly for *E. coli* and *Klebsiella pneumoniae*. Gram-positive bacteria, especially MRSA and *Enterococcus* spp., exhibited significant resistance to Fluoroquinolones but retained sensitivity to Linezolid. However some of the isolates of *Enterococci* showed resistance to Vancomycin. The study concludes that judicious use of antibiotics, especially in OPD settings, will influence the overall drug resistance pattern in the community. This study emphasizes the need for antimicrobial surveillance at the local and regional areas.

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