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A Study on Multidrug-Resistant Gram-Negative Bacilli from Urinary Samples in Patients of a Tertiary Care Centre

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ABSTRACT

Urinary tract infections (UTIs), caused by microbial overgrowth in the urinary system, result in symptoms like fever, painful urination, and abdominal discomfort. Common pathogens include E. coli, Klebsiella pneumoniae, and Staphylococcus species, with rising antibiotic resistance posing a major health challenge. This study focuses on UTI cases at KR Hospital, Mysore, to track antimicrobial resistance trends. Conducted over six months (Sept 2024 - Feb 2025) at Mysore Medical College, the study involved adult and pediatric patients with suspected UTIs. Urine samples were cultured on Mac'Conkey, CLED, and blood agar to identify bacteria, with counts ≥10^5/mL indicating infection. Antimicrobial susceptibility was assessed using the Kirby–Bauer method, and multidrug-resistant (MDR) isolates were further tested for ESBL and MBL production. Data were analysed using SPSS. Out of 320 samples, 250 were positive for bacterial growth, with the highest number of MDR isolates in those aged 36-50. UTI prevalence was highest among females and those over 60, aligning with previous research. The study found 78.12% of isolates to be multidrug-resistant, with E. coli and Klebsiella pneumoniae as the primary pathogens. The study revealed major resistance to cephalosporins and penicillins, but sensitivity to colistin. Among MDR organisms, 28.8% were ESBL producers, and 12% were MBL producers, with the highest rates of ESBL production found in E. coli and among the 36-50 age group. Resistance was notable for cephalosporins and penicillins, but sensitivity was preserved for amikacin, gentamicin, piperacillin-tazobactam, cotrimoxazole, tetracycline and fosfomycin.

Keywords: *Urinary Tract Infection; Antimicrobial Resistance; Escherichia coli; Multidrug-Resistant Bacteria*

INTRODUCTION

Urinary tract infections (UTIs) occur due to the microbial overgrowth within the urinary system. Common symptoms associated with UTIs include fever, dysuria, burning micturition, increased frequency of urination, and lower abdominal pain; these infections can potentially lead to lasting kidney damage. UTIs are classified as either community-acquired or hospital-acquired. They may present with or without symptoms and pose a significant burden on public health systems while adversely affecting quality of life[1]. Bacteria typically

inhabit the area around the urethra and can colonize the bladder but are usually expelled during urination. Women are more susceptible to UTIs than men due to the closer proximity of their bladder to the urethral opening, which is also near the rectum. Additionally, urogenital practices or medical procedures can facilitate bacterial movement towards the urethra[2].

The most common pathogen responsible for UTIs is Escherichia coli, followed by Klebsiella pneumoniae, various species of Staphylococcus, Proteus species, Pseudomonas aeruginosa, Enterococcus species, and Enterobacter sp.[1] Approximately 150 million cases of UTI are diagnosed annually, incurring healthcare costs exceeding $6 billion[1]. While antibiotic sensitivity data helps in selecting empirical treatments for UTIs, such information primarily concerns complex cases. As a result, empirical treatment is often employed in areas lacking urine culture facilities, leading to unnecessary antibiotic use. The rising prevalence of drug-resistant uropathogens is a serious public health challenge that underscores the need for ongoing screening for antibiotic susceptibility among UTI-causing organisms. The patterns of antimicrobial sensitivity fluctuate over time and across different geographical areas. Hence, it is crucial to conduct susceptibility screenings at each location to generate current epidemiological data. Since it is the largest tertiary care hospital attached to Mysore Medical College catering the needs of thousands of people from southern districts of Karnataka, the present study will be carried out among patients admitted at KR Hospital.

MATERIALS AND METHODS

The current study was carried out in the Department of Microbiology at Mysore Medical College and Research Institute for a period of 6 months from September 2024 to February 2025.In this study, urine samples of patients with clinically suspected UTI were collected and tested. Both male and female - adult and paediatric patients having symptoms of UTI (burning micturition, increase in frequency, urgency, pain above pubic symphysis) were included and all patients with history of hospitalization (1 week prior to presentation, or more than 48 h), known cases of neurogenic bladder, obstructive uropathy or vesicoureteral reflex and on antibiotic prophylaxis were excluded from this study. Details like age, sex and comorbidities were collected from each patient.

Midstream clean-catch urine samples were aseptically obtained in wide-mouth sterile containers before any antibiotic therapy commenced. Samples were processed within an hour at the microbiology laboratory. Macroscopic examination assessed colour and turbidity; wet mounts were prepared for microscopic evaluation. A modified semi-quantitative approach using a standard wire loop (0.01 ml) was utilized for inoculating MacConkey agar plates as well as CLED agar and blood agar plates which were then incubated aerobically at 37°C for 24 hours. Later colonies were counted: a bacterial count equal to or greater than 10^5 per ml was deemed significant for diagnosing UTI; counts between 10^2-10^4 per ml indicated suspected bacteriuria while counts below 10^2 per ml reflected non-significant bacterial growth.

Identification of bacterial isolates adhered to established microbiological methods based on culture characteristics and biochemical analyses. Antimicrobial susceptibility testing was performed on Mueller-Hinton agar utilizing the Kirby-Bauer disk diffusion method under Clinical Laboratory Standards Institute (CLSI) guidelines' recommendations. Antibiotics evaluated against Gram-negative bacilli encompassed ampicillin; amoxicillin/clavulanic acid; piperacillin/tazobactam; cefepime; ceftazidime; ceftriaxone; cefuroxime; imipenem; meropenem; ertapenem; amikacin; gentamicin; ciprofloxacin; fosfomycin; nitrofurantoin; co-trimoxazole; aztreonam; and colistin. Multidrug-resistant isolates underwent additional screening for

ESBL and MBL production through combination disks containing ceftazidime/ ceftazidime-clavulanate and imipenem/ imipenem with EDTA respectively.

Upon completion of data collection procedures all information was entered into SPSS version 25 for statistical analysis purposes where quantitative variables were summarized via their mean values alongside standard deviations while qualitative variables observed percentages accordingly corresponding bivariate analyses alongside relevant statistical tests being conducted.

RESULT

In the study total 320 samples were collected, out of which 250 samples tested positive for bacteriological analysis. 145 samples were from females and 105 samples were from males. 16 cultures were positive for multi drug resistant organisms among 0 to 5 age group, 27 for 6 to 10 years, 18 for 11 to 14 years, 51 for 15 to 35 years 75 for 36 to 50 years and 36 for 71 to 100 years similar to findings by Luty et al (2021).

In the current study, burning micturition was observed as the commonest symptom (37.6%) followed by frequency (30.4%), urgency (29.1%), suprapubic pain (23%) and dribbling of urine (6.5%). Most of the patients were diabetic (58%).

Escherichia coli was the major multidrug resistant pathogen, isolated from 114 samples (45.6%) followed by Klebsiella species from 94 samples (37.6%)[3,9,10,11,12] similar to findings by Gahlot et al. Other uropathogens were Proteus species from 16 samples (6.4%), Acinetobacter species from 9 samples (3.6%), Enterobacter species from 8 samples (3.2%), Pseudomonas species from 5 samples (2%) and Citrobacter species from 4 samples (1.6%)- Table 1.

Table 1 – Proportion of Gram-Negative pathogens isolated from urinary samples

ORGANISM	NO OF ISOLATES (n=250)	PERCENTAGE
E Coli	114	45.6
Klebsiella species	94	37.6
Enterobacter species	8	3.2
Proteus species	16	6.4
Pseudomonas species	5	2
Citrobacter species	4	1.6
Acinetobacter species	9	3.6

AST pattern showed resistance of majority of organisms isolated to Cephalosporins- Cefotaxime (240), Ceftriaxone (211), Cefuroxime (236), Cefepime (178), Ceftazidime (245) followed by Penicillins – Ampicillin (224), Quinolones-Ciprofloxacin (176) , Beta lactam -Beta lactam Inhibitors – Amoxycillin Clavulanate (81),Piperacillin - Tazobactam(11), Aminoglycosides – Amikacin (5), Gentamicin (5) and Carbapenems – Imipenem (75), Meropenem (75), Ertapenem (74), Monobactams- Aztreonam (152), Tetracycline (36) and Nitrofurantoin (89) – Table 2. No drug resistance was observed with Colistin and Ampicillin-Sulbactam.

Drug resistance shown by major pathogens Escherichia coli and Klebsiella species are shown in Tables 3 & 4. Escherichia coli showed resistance majorly towards cephalosporins and penicillins followed by carbapenems while Klebsiella species showed major resistance to cephalosporins followed by penicillins and aminoglycosides. None of the pathogens were resistant to Colistin.

Table 2- Drug resistance pattern observed among all the multidrug resistant Gram Negative pathogens

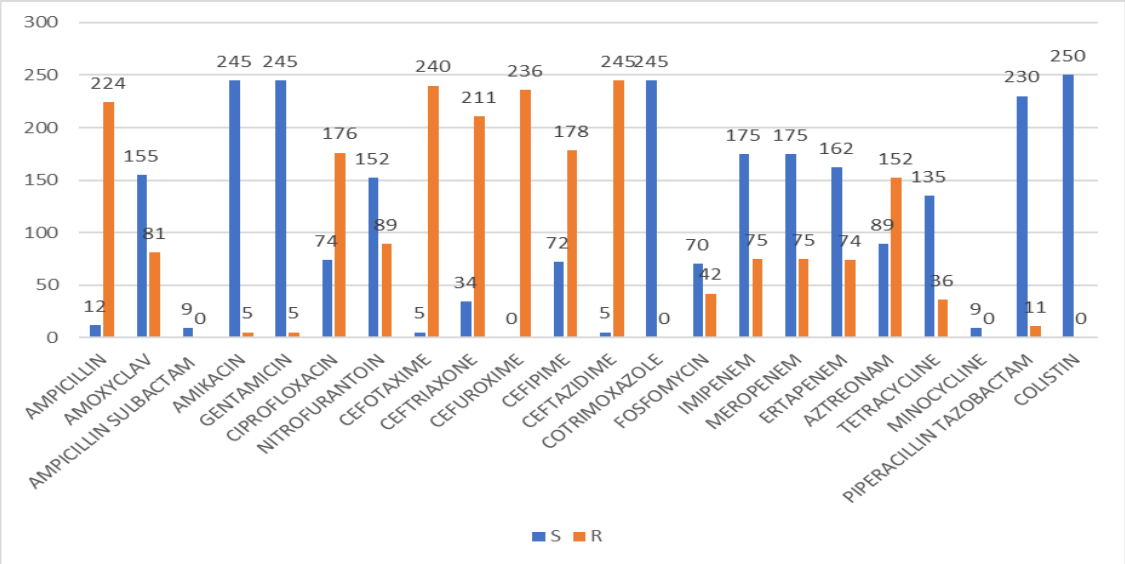


Table 3- Drug resistance pattern observed in E.Coli isolated from urine samples.

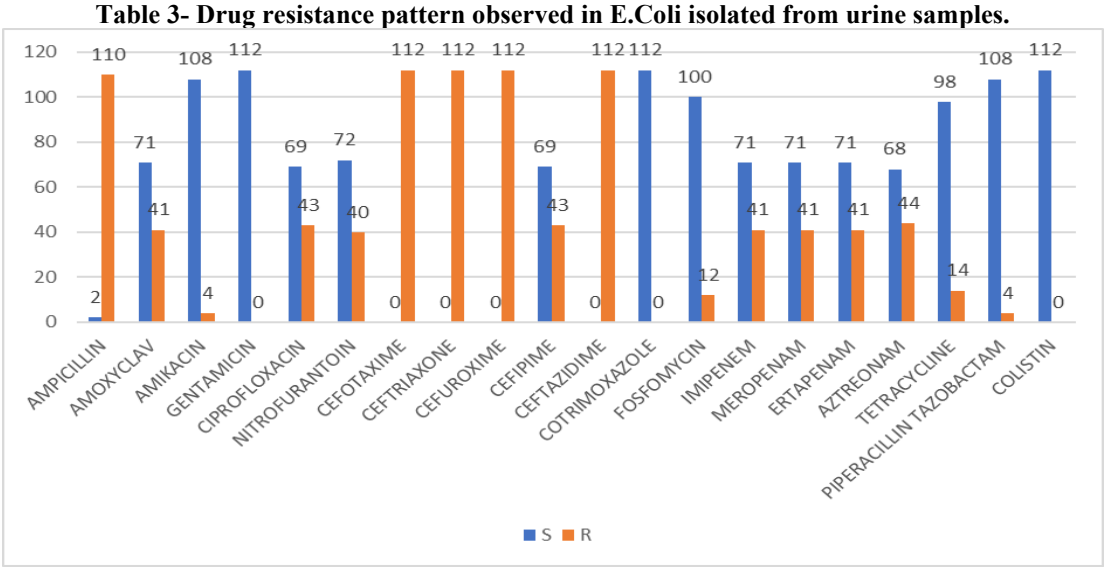
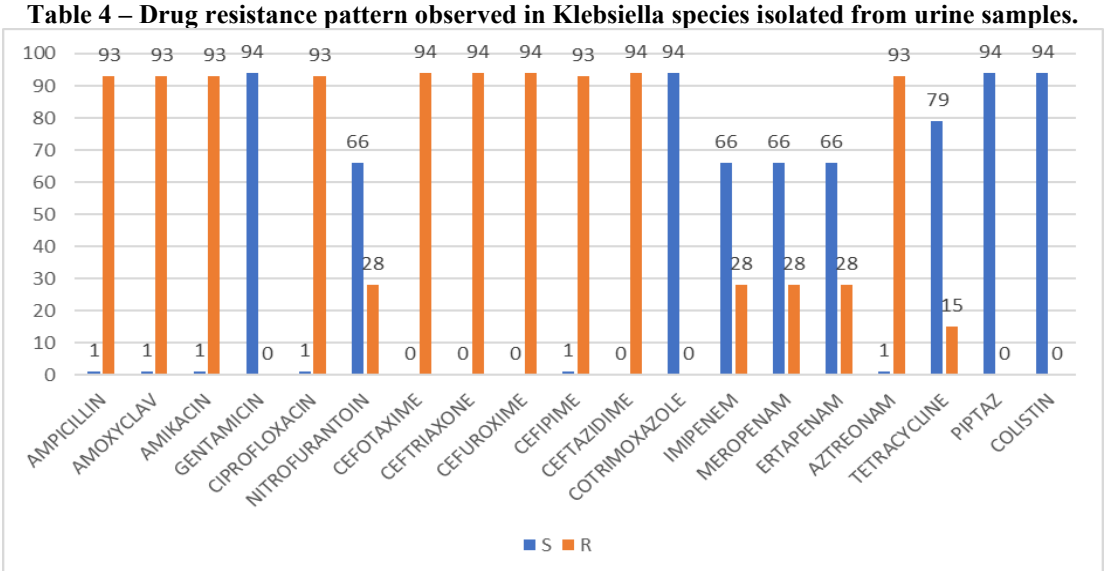
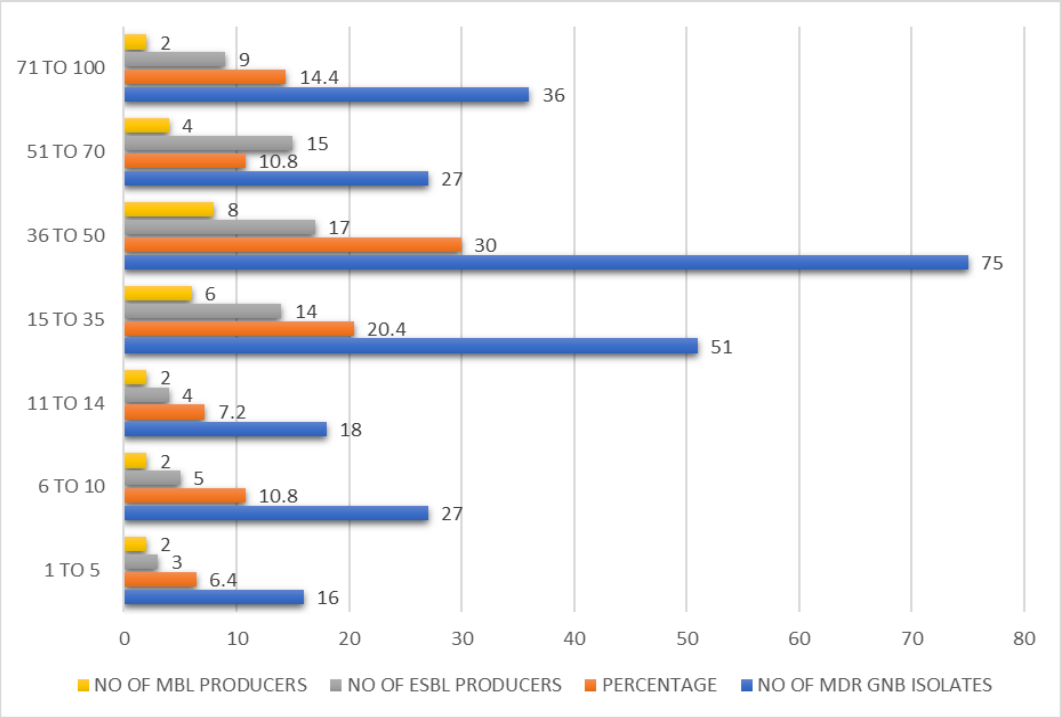


Table 4 – Drug resistance pattern observed in Klebsiella species isolated from urine samples.



Among the 250 Multidrug resistant organisms, ESBL producers were 72(28.8%) while MBL producers were 30(12%). Majority of ESBL producers were seen from the isolates belonging to age group 36 to 50 years followed by 15 to 35 years and 51 to 70 years -Table 5. 41 females and 29 males had UTI caused by ESBL producing pathogens while 21 females and 8 males had UTI caused by MBL producing pathogens. 39 isolates of E. coli (34.8%), 25 isolates of Klebsiella species (26.5%),3 isolates of Enterobacter species (37.5%),2 isolates each of Acinetobacter species and Citrobacter species and 1 isolate from Pseudomonas species and Proteus species exhibited ESBL production.17 isolates of E. coli (15.12%) ,10 isolates of Klebsiella species (10.6%) and 1 isolate each from Enterobacter species and Citrobacter species exhibited MBL production.

Table 5 – Age wise distribution of multidrug resistant organisms



DISCUSSION

Antimicrobial resistance among uropathogens is increasing globally and considered to be a challenge for clinicians since there are limited treatment options. Management of UTIs in the era of antimicrobial resistance requires a systematic approach to diagnose the type of infection and to select appropriate antimicrobial agent.

This study analyzed 320 urine samples, of which 250 yielded positive cultures, indicating a high burden of urinary tract infections (UTIs) in the study population. The majority of isolates were from females (145), supporting previous findings that women are at higher risk for UTIs due to anatomical and physiological factors[3]. The age-wise distribution showed the highest prevalence of multidrug-resistant (MDR) organisms in the 36 to 50 years group, followed by the 15 to 35 years and 71 to 100 years groups. These findings are consistent with earlier studies, including those by Luty et al., who reported similar age-based clustering of resistance[3,4].

Symptomatically, burning micturition (37.6%) was the most common complaint, followed by increased frequency (30.4%), urgency (29.1%), suprapubic pain (23%), and dribbling of urine (6.5%)[1]. Additionally, the high prevalence of diabetes (58%) among patients highlights the well-established link between diabetes mellitus

and increased susceptibility to UTIs, especially with MDR pathogens [5,6,7].

The most frequently isolated organism was *Escherichia coli* (45.6%), followed by *Klebsiella* species (37.6%), which aligns with numerous other Indian and international studies[8-11,15,16]. Other uropathogens such as *Proteus*, *Acinetobacter*, *Enterobacter*, *Pseudomonas*, and *Citrobacter* were isolated in smaller proportions, echoing previous reports on the polymicrobial nature of UTIs, particularly in complicated and nosocomial cases[11-14].

Antimicrobial susceptibility testing (AST) revealed alarmingly high levels of resistance to commonly used antibiotics. Most isolates exhibited resistance to cephalosporins such as cefotaxime, ceftriaxone, cefuroxime, cefepime, and ceftazidime. Similarly, penicillin derivatives like ampicillin and beta-lactam/beta-lactamase inhibitor combinations like amoxicillin-clavulanate and piperacillin-tazobactam showed reduced efficacy. Notably, resistance to ciprofloxacin was also high, underlining the declining usefulness of fluoroquinolones in empirical UTI management. Amikacin and gentamicin (aminoglycosides) and nitrofurantoin retained better activity, consistent with findings from earlier studies suggesting their continued utility in treating MDR infections[7]. The low resistance to colistin is reassuring but must be interpreted with caution due to the potential for nephrotoxicity and the risk of emerging resistance when used indiscriminately.

Among the 250 MDR isolates, 28.8% were extended-spectrum beta-lactamase (ESBL) producers, and 12% were metallo-beta-lactamase (MBL) producers. These findings reflect the global surge in beta-lactamase-mediated resistance mechanisms, particularly among *E. coli* (34.8% ESBL, 15.12% MBL) and *Klebsiella* species (26.5% ESBL, 10.6% MBL)[12-15]. Other species, such as *Enterobacter*, *Acinetobacter*, *Citrobacter*, and *Proteus*, also contributed to ESBL/MBL positivity, though in smaller numbers. The predominance of ESBL production in the 36 to 50 years age group could reflect higher antibiotic exposure and comorbidity burden in this demographic.

The gender-wise distribution of ESBL- and MBL-producing pathogens showed a higher prevalence in females, mirroring the overall gender distribution in culture-positive UTIs. These resistance trends underscore the importance of culture-guided therapy in managing UTIs, particularly in tertiary care settings where MDR organisms are prevalent[16].

CONCLUSION

The findings of our study highlight the pressing need for routine antimicrobial susceptibility testing, antimicrobial stewardship programs, and infection control measures. The high prevalence of MDR and beta-lactamase producing uropathogens limits empirical therapy options and stresses the necessity for region-specific antibiograms. The results of this study are in accordance with several previous studies. Our study has some limitations as the isolates were collected only from a single centre. These results cannot be easily used to generalize findings from studies with a larger sample size.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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