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Original Research Article

Antimicrobial resistance patterns of *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with urinary tract infections: a prospective study from a tertiary care hospital in Hyderabad

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ABSTRACT

Background: Urinary tract infections (UTI) are among the most prevalent healthcare-associated infections (HAI), with *E. coli* and *K. pneumoniae* being the leading etiological agents. The increasing trend of antimicrobial resistance among these uropathogens threatens effective treatment, necessitating regular local surveillance. Therefore, the objective of this investigation is to assess the antibiotic resistance pattern among urinary isolates of *E. coli* and *K. pneumoniae.*
Methods: A prospective observational study was conducted from January to April 2025 in the department of microbiology at Apollo Institute of Medical Sciences and Research, Hyderabad. A total of 305 non-duplicate urine samples from UTI patients were processed. Isolates of *E. coli* and *K. pneumoniae* were identified using standard microbiological procedures. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to CLSI guidelines.
Results: Of the 305 samples, 88 (28.85%) were culture-positive for *E. coli* (60.2%) and *K. pneumoniae* (18.2%). *E. coli* isolates were predominantly recovered from patients aged 60–80 years and females (73.58%). *K. pneumoniae* was more frequent in the 20-40 years' age group and also showed a female predominance (56.25%). High resistance was observed to third-generation cephalosporins, especially among *E. coli*. However, *E. coli* showed high susceptibility to fosfomycin (100%), nitrofurantoin (94%), and amikacin (92%). *K. pneumoniae* exhibited lower sensitivity overall, with highest susceptibility to amikacin (75%) and cotrimoxazole (75%).
Conclusions: The study represents *E. coli* and *K. pneumoniae* as major uropathogens with concerning resistance patterns, particularly to third-generation cephalosporins. These findings reinforce the need for routine antimicrobial resistance monitoring and evidence-based empirical therapy to optimize UTI management.

Keywords: Uropathogens, Antimicrobial resistance, *E. coli, Klebsiella pneumoniae,* UTI

INTRODUCTION

Healthcare-associated infections (HAI) face significant challenges in clinical management. UTI are the most frequently encountered HAI that impedes optimal patient care outcomes. It is defined as proliferation and invasion of microorganisms in any part of urinary tract, from the renal cortex to the urethral meatus.[1] UTIs are particularly prevalent among young, sexually active, premenopausal women.[2] Among infections of the urinary tract involve the kidneys (pyelonephritis), bladder (cystitis) and urethra (urethritis). UTIs are associated with short-term health issues such as fever, painful urination, and lower abdominal discomfort. It can lead to complications like permanent kidney scarring.[3] They are among the most widespread infections globally, contributing significantly to healthcare costs, mortality and morbidity.[4]

Although a number of bacteria can cause UTIs, gram negative bacteria-especially *E. coli* are the most prevalent uropathogens and cause majority of infections in different settings. *P. aeruginosa, Proteus*, and *Klebsiella* are the less prevalent gram-negative, but some gram-positive bacteria, such as *S. saprophyticus* and *Streptococcus* spp. have been implicated with UTI.[5] The rising prevalence of antibiotic resistance is limiting the available treatment options for UTI.[6] Gram-negative bacteria's resistance to antibiotics is mediated by a number of mechanisms like enzymatic activation, reduced permeability across the cell wall as a result of porin deficiency, and efflux pumps.[7]

Broad-spectrum antibiotics are commonly employed for empirical treatment because of their proven success in treating UTIs. However, the indiscriminate use of these drugs often without proper medical supervision and facilitated by over-the-counter accessibility, which has significantly contributed to the emergence and spread of multidrug resistance (MDR) in common infectious diseases worldwide.[8] The epidemiology, types of species, and resistance patterns of uropathogens showed significant variation across different geographical regions and population.[9] Grasping implications of drug resistance is crucial, as shifting patterns in antibiotic resistance significantly influence the choice of empirical treatment for UTIs.

Regular surveillance is essential for developing sophisticated and evidence-based empirical antimicrobial therapy guidelines. It ensures treatments remain effective against evolving resistance patterns, helps prevent treatment failures, and supports responsible antibiotic use.

Therefore, the aim of the study is to understand the resistance pattern among the urinary isolates of *E. coli* and *K. pneumoniae*.

METHODS

This four-month (January 2025 to April 2025) study was carried out at the Apollo Institute of medical sciences and research's department of microbiology in Hyderabad, Telangana. Apollo hospital, Hyderabad is a 660-bed setting that provides the public with high-quality healthcare. This retrospective analysis study included 305 non-repeated urine samples from both outpatient and inpatients of UTI whose urine cultures isolated showed significant bacterial growth. The exclusion criteria included specimens and bacterial isolates obtained from the same patient on multiple occasions to prevent duplication of individual cases. Additionally, incomplete records and improperly labeled or contaminated samples were also excluded to maintain the integrity and quality of data.

The samples were collected aseptically and transported immediately to the laboratory within two hours. After being cultured on blood agar and MacConkey agar, Gram stain followed by oxidase, catalase and other biochemical assays were carried out to identify the isolates of *E. coli* and *K. pneumoniae* using standard microbiological guidelines.

After the initial identification of the isolates, Mueller Hinton agar was used for antimicrobial susceptibility testing using the Kirby Bauer disc diffusion method as per the CLSI guidelines. A suspension of test and control organisms were made to correspond to the optical density of 0.5 McFarland turbidity. Following this, the Mueller Hinton agar plates were inoculated with the suspension and the antibiotic discs were positioned on the culture surface, 20 mm from center to center after the plate had dried. Antibiotics were gentamicin 120 µg, amikacin 10 µg, ceftazidime 30 µg, cefuroxime 30 µg, ceftriaxone 30 µg, ciprofloxacin 5 µg, ofloxacin 5 µg, cotrimoxazole 25 µg, piperacillin/ tazobactam 100/10 µg, cefoperazone/ sulbactam 75/10 µg, meropenem 10 µg, imipenem 10 µg, cefepime 30 µg, cefazolin 30 µg, nitrofurantoin 300 µg, fosfomycin 200 µg, cefoxitin 30 µg, cefotaxime 30 µg. For 15-23 hours, the plates were incubated at 37°C. The presence of inhibition zone around the disc determines the organism's susceptibility to the antibiotic.

Statistical analysis

Microsoft excel (Microsoft Corp., Redmond, WA, US) was used to enter the data, and GraphPad Instat (Insight Venture Management, LLC, New York, NY, US) was used to perform the Chi-square test. Following analysis,the data were displayed as p-values and percentages. If the results probability was less than 0.05, it was considered statistically significant.

RESULTS

Out of 305 urine samples that were collected from patients presenting with UTI, 88 (28.85%) were *E. coli and K. pneumoniae* isolated from cultures. Out of the 88 urine samples that showed significant bacteria growth, *E. coli* was the most frequently isolated uropathogen, being identified in 53 samples, which constitutes 60.2% of the total isolates. *K. pneumoniae* were the second most common, isolated in 16 samples, accounting for 18.2%. The remaining 19 samples, representing 21.6% yielded other bacterial pathogens. This distribution highlights *E. coli* as the predominant causative agent of UTI in the studied population, followed by *K. pneumoniae.*

The age-based distribution of *E. coli* and *K. pneumoniae* isolates show variation across different age groups as seen in the Table 1 below. *E. coli* was most frequently isolated from patients aged 60-80 years, accounting for 35.85%, followed by 20-40 years group at 28.30%. A smaller proportion was observed in the 40-60 years group (24.53%) and the lowest in 0-20 years group (11.32%). In contrast, highest frequency of *K. pneumoniae* was in 20-40 years group (37.5%), followed by equal distribution in the 40-60 years and 60-80 years group (31.25%).

The distribution of *E. coli* and *K. pneumoniae* isolates based on gender as shown in Table 2, represents a higher prevalence among female patients in case of both the isolates. For *E. coli*, 73.58% of isolates were obtained from females while only 26.42% were from males. Similarly, *K. pneumoniae* was more frequently isolated from female patients (56.25%) compared to male (43.75%). This suggests a female predominance in the isolation of both organisms in the studied population.

Table 3 reveals marked differences in antibiotic resistance patterns between *E. coli* and *K. pneumoniae* isolates. The Chi-square test yielded a value of 463.68 with a p<0.0001, indicating statistical significance. Among *E. coli* isolates, high sensitivity was observed for fosfomycin (100%), nitrofurantoin (94%), amikacin (92%) and piperacillin/tazobactam (85%) indicating these as effective treatment options. Conversely, resistance was notably high against third-generation cephalosporins such as cefuroxime (91%), ceftriaxone (87%) and ceftazidime (85%). *Klebsiella* isolates showed lesser sensitivity across most antibiotics compared to *E. coli*, with highest susceptibility to amikacin (75%) and cotrimoxazole (75%), while resistance was prominent to cefoxitin (75%), cefuroxime (63%), nitrofurantoin (69%).

These findings highlight urgent need for tailored antibiotic stewardship strategies based on local resistance trends.

Table 1: Age-based distribution of *E. coli* and *K. pneumoniae.*

Age (in years)	*E. coli*, N (%)	*Klebsiella pneumoniae*, N (%)
0-20	6 (11.32)	0
20-40	15 (28.30)	6 (37.5)
40-60	13 (24.53)	5 (31.25)
60-80	19 (35.85)	5 (31.25)

*n=Number of isolates in each age group, %= Percentage of total isolates found in that age group.

Table 2: Gender-based distribution of *E. coli* and *K. pneumoniae.*

Gender	*E. coli*, N (%)	*K. pneumoniae*, N (%)
Female	39 (73.58)	9 (56.25)
Male	14 (26.42)	7 (43.75)
Total	53 (100)	16 (100)

*n=Number of isolates within each gender category, %=Percentage of total isolates found in that gender category.

Table 3: Antimicrobial Susceptibility pattern among *E. coli* and *K. pneumoniae.*

Variables	*E. coli*, N (%)		*K. pneumoniae*, N (%)	
	Sensitive	Resistance	Sensitive	Resistance
Gentamicin 120 µg	40 (75)	13 (25)	9 (56)	7 (44)
Amikacin 10 µg	49 (92)	4 (8)	12 (75)	4 (25)
Ceftazidime 30 µg	8 (15)	45 (85)	5 (31)	11 (69)
Cefuroxime 30 µg	5 (9)	48 (91)	6 (38)	10 (63)
Ceftriaxone 30 µg	7 (13)	46 (87)	7 (44)	9 (56)
Ciprofloxacin 5 µg	16 (30)	37 (70)	6 (38)	10 (63)
Ofloxacin 5 µg	18 (34)	35 (66)	7 (44)	9 (56)
Cotrimoxazole 25 µg	25 (47)	28 (53)	12 (75)	4 (25)
Piperacillin/ tazobactam 100/10 µg	45 (85)	8 (15)	11 (69)	5 (31)
Cefoperazone/ sulbactam 75/10 µg	38 (72)	15 (18)	10 (63)	6 (38)
Meropenem 10 µg	44 (83)	9 (17)	11 (69)	5 (31)
Imipenem 10 µg	42 (79)	11 (20.75)	11(69)	5 (31)
Cefepime 30 µg	11 (21)	42 (79)	8 (50)	8 (50)
Cefazolin 30 µg	8 (15)	45 (85)	5 (31)	11 (69)
Nitrofurantoin 300 µg	50 (94)	3 (6)	5 (31)	11 (69)
Fosfomycin 200 µg	53 (100)	0	5 (31)	11 (69)
Cefoxitin 30 µg	7 (13)	46 (87)	4 (25)	12 (75)
Cefotaxime 30 µg	6 (11)	47 (89)	6 (38)	10 (62)

*$\chi 2$=463.68, p<0.0001, significant. µg=Microgram, n=Number of isolates showing either resistance or sensitivity to that antibiotic, %=Percentage of total isolates found in that gender category, $\chi 2$=chi square test.

DISCUSSION

The present study highlights *E. coli* and *K. pneumoniae* as significant uropathogens isolated from urine cultures of patients with UTI, with *E. coli* accounting for 60.2% and *K. pneumoniae* 18.2% of total isolates. This is consistent with earlier research indicating *E. coli* as the predominant causative agent of UTIs globally, owing to its uropathogenic virulence factors and ability to colonize the urinary tract effectively.[10,11]

The age-based distribution showed that *E. coli* was more prevalent in the elderly population (60-80 years), while *K. pneumoniae* showed a higher occurrence in the 20-40 years age group. These findings align with prior reports suggesting that advanced age is associated with increased risk of UTI due to comorbidities and catheterization.[12] Gender-based, the isolation of both organisms was more frequent among females, particularly for *E. coli* (73.58%). This is attributable to anatomical and physiological factors in females that predispose them to UTIs.

Antimicrobial susceptibility testing revealed high resistance rates among *E. coli* and *K. pneumoniae* to third-generation cephalosporins such as ceftazidime, cefotaxime, and cefuroxime. This resistance is likely due to extended spectrum β-lactamase (ESBL) production, as observed in similar regional studies.[13] *E. coli* isolates exhibited excellent susceptibility to fosfomycin (100%), nitrofurantoin (94%) and amikacin (92%), corroborating findings from studies by Rizvi et al.[14]

K. pneumoniae isolates demonstrated comparatively lower sensitivity across most antibiotics, although notable susceptibility was seen with amikacin (75%) and cotrimaxazole (75%). The highest resistance observed in *K. pneumoniae* may reflect its increasing role is nosocomial infections and its known propensity for acquiring MDR.[15]

These results emphasize the importance of empirical therapies for UTIs and the need for strict vigilance regarding the uropathogens, *E. coli* and *K. pneumoniae* due to its high resistance profile. Ongoing antimicrobial stewardship and periodic resistance monitoring remain imperative to improve clinical outcomes.

This study was carried out at a single tertiary care hospital over a defined time period, which may influence the extent to which the findings can be generalized to other settings or populations. The study utilized standard phenotypic methods which are widely accepted and practical in routine clinical microbiology laboratories but may limit the study. However, inclusion of molecular techniques-such as PCR-based detection of resistance genes, could further enhance the accuracy, sensitivity and specificity of results. Molecular methods also allow for better epidemiological tracking and deeper insight into the genetic basis of resistance. Despite this, the current findings provide meaningful data on local antimicrobial resistance trends and remain valuable for guiding empirical treatment strategies and informing infection control policies in similar healthcare environments.

CONCLUSION

This study underscores the predominance of *E. coli* and *K. pneumoniae* as key uropathogens in urinary tract infections, with *E. coli* being more prevalent, especially among elderly females. Alarmingly, in both organisms, pointing towards widespread ESBL production. While *E. coli* retained high susceptibility to fosfomycin, nitrofurantoin and amikacin, *K. pneumoniae* showed comparatively lower sensitivity, reflecting its multidrug-resistant nature. These findings highlight the urgent need for regular surveillance and localized antibiotic stewardship programs to guide effective empirical therapy and combat rising resistance trends in uropathogens.

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