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RESEARCH ARTICLE

Isolation, Identification and Antibiotic Susceptibility Pattern of Multidrug-Resistant *Klebsiella pneumoniae* from Urine Samples in a Tertiary Care Hospital

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ABSTRACT

Klebsiella pneumoniae is one of the leading causes of urinary tract infections (UTIs) and is increasingly associated with multidrug resistance, posing significant therapeutic challenges. Continuous surveillance of its antimicrobial susceptibility profile is essential for effective clinical management. This cross-sectional study was conducted from July 2023 to December 2023 in a tertiary care hospital. A total of 12 urine samples were collected from patients with suspected UTIs. Samples were cultured using standard microbiological techniques, and isolates were identified by colony morphology, Gram staining, and biochemical tests. Antibiotic susceptibility testing was performed by the Kirby–Bauer disc diffusion method following CLSI guidelines. Out of 12 urine samples, 10 (83%) showed bacterial growth and were identified as *Klebsiella pneumoniae*. Among the isolates, 8 (80%) were from female patients and 2 (20%) from male patients. The majority of patients (40%) belonged to the 41–60 years age group, followed by ≥61 years (30%). Most isolates were obtained from OPD patients (70%), while 30% were from IPD patients. The highest sensitivity was observed to Meropenem (90%) and Tigecycline (90%), followed by Gentamicin (70%). Moderate sensitivity (50%) was noted for Cefotaxime, Amoxicillin, Ceftazidime, Cefoxitin, Ampicillin, and Ciprofloxacin. The lowest sensitivity was seen with Doxycycline (10%) and Colistin (40%). Multidrug resistance (MDR) was detected in 7 (70%) isolates, while no

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extensively drug-resistant (XDR) or pan drug-resistant (PDR) strains were identified. *Klebsiella pneumoniae* remains a significant uropathogen with a high prevalence of multidrug resistance. Carbapenems and Tigecycline showed the highest effectiveness, whereas resistance to commonly used antibiotics was considerable. Regular antimicrobial surveillance and rational antibiotic use are essential to control the spread of resistant strains.

Keywords: *Klebsiella pneumoniae*, UTI, MDR, Antibiotic Susceptibility, AMR

1. Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases worldwide and represent a major public health burden. They affect individuals of all age groups and are particularly frequent among women, elderly individuals, hospitalized patients, and immunocompromised populations[1]. UTIs contribute substantially to patient morbidity, increased healthcare costs, and increased workload within healthcare systems. Early and accurate diagnosis, along with appropriate antimicrobial therapy, is essential to reduce complications, prevent recurrence, and limit the spread of resistant organisms[2].

UTIs are caused by a wide range of microbial pathogens, including Gram-negative and Gram-positive bacteria, and occasionally fungi[3]. Among these, members of the Enterobacteriaceae family are the predominant causative agents. *Klebsiella pneumoniae* has emerged as one of the most significant uropathogens, frequently reported as the second most common cause of UTIs after *Escherichia coli* in many clinical settings. *Klebsiella pneumoniae* is a Gram-negative, lactose-fermenting, non-motile, encapsulated bacillus that is widely distributed in the environment and can colonize the human gastrointestinal tract[4]. Although it may exist as part of the normal flora, it possesses considerable pathogenic potential and is capable of causing various infections, including urinary tract infections, pneumonia, septicemia, and wound infections[5].

The virulence of *Klebsiella pneumoniae* is attributed to multiple factors such as its polysaccharide capsule, lipopolysaccharide, siderophore production, adhesins, and biofilm-forming ability[6]. These virulence determinants enhance bacterial survival, facilitate colonization, and contribute to increased morbidity and mortality. In recent years, the rapid emergence of multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL)-producing strains has become a serious global concern[7]. The irrational and excessive use of antibiotics in both hospital and community settings has accelerated the development of resistance, significantly narrowing available therapeutic options and complicating clinical management[8].

Antimicrobial resistance among urinary pathogens varies geographically and over time, emphasizing the importance of continuous surveillance and local antimicrobial susceptibility profiling. Knowledge of current resistance trends is essential for selecting appropriate empirical therapy and implementing effective antibiotic stewardship programs[9].

Therefore, the present study was undertaken to isolate and identify *Klebsiella pneumoniae* from urine samples of patients with urinary tract infections and to determine their antibiotic susceptibility patterns in order to provide updated information for better clinical management and control of resistant strains.

2. Methodology

This cross-sectional study was conducted in the Microbiology Laboratory of Bangladesh University of Health Sciences (BUHS) over a period of six months, from July 2023 to December 2023. The study population included patients attending both the outpatient department (OPD) and inpatient department (IPD) with clinical suspicion of urinary tract infection (UTI). Patients were selected based on clinical symptoms and medical history suggestive of UTI. Only patients who provided informed written consent were included in the study, while those unwilling to participate were excluded. Ethical considerations were strictly maintained, and confidentiality of patient information was ensured throughout the study.

Demographic and clinical data were collected at the time of enrollment using a pre-designed data collection form. Relevant patient information, including clinical findings and laboratory reports, was recorded systematically. Data were later analyzed using the Statistical Package for Social Sciences (SPSS).

Urine samples were collected following standard microbiological procedures. Patients were instructed to wash their hands thoroughly before specimen collection. A clean, dry, sterile, wide-mouthed container was provided, and midstream clean-catch urine was collected to minimize contamination. Patients were instructed to begin urinating into the toilet, collect the midstream portion in the sterile container, and then finish voiding into the toilet. In cases where patients were unable to provide a clean-catch specimen, catheterized urine samples were collected using strict aseptic technique. All samples were transported to the laboratory within two hours of collection. If processing was delayed, specimens were preserved at 2–8°C but not for more than 24 hours[10].

Upon receipt in the laboratory, specimens were properly labeled and recorded. Urine samples were inoculated onto MacConkey agar and Blood agar (or Brain Heart Infusion agar) using standard loop techniques and incubated aerobically at 37°C for 18–24 hours. After incubation, plates were examined for bacterial growth, and colony characteristics were observed. Suspected colonies were further processed for identification through Gram staining and standard biochemical tests[11].

Identification of *Klebsiella pneumoniae* was based on colony morphology, Gram staining, and biochemical reactions. On MacConkey agar, the organism produced large, mucoid, pink lactose-fermenting colonies. Gram staining revealed Gram-negative bacilli. Biochemical tests including oxidase, catalase, citrate utilization, bile esculin hydrolysis, MIU (Motility, Indole, Urease), and Triple Sugar Iron (TSI) tests were performed. The isolates were oxidase-negative, citrate-positive, bile esculin-positive, non-motile, indole-negative, and showed slow urease positivity. TSI reaction showed acid slant and acid butt with gas production and no hydrogen sulfide[12].

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines. A pure colony was suspended in sterile normal saline, and the turbidity was adjusted to 0.5 McFarland standard. The bacterial suspension was uniformly inoculated onto Mueller-Hinton agar plates using a sterile cotton swab. Antibiotic discs were then placed on the agar surface and incubated at 37°C for 18–24 hours. The antibiotics tested included Cefotaxime (30 µg), Amoxicillin (10 µg), Ceftazidime (30 µg), Cefoxitin (30 µg), Meropenem (10 µg), Tigecycline (15 µg), Doxycycline (30 µg), Colistin (10 µg), Ampicillin (10 µg), Gentamicin (10 µg), and Ciprofloxacin (5 µg). After incubation, zones of inhibition were measured in millimeters and interpreted as sensitive, intermediate, or resistant according to CLSI standards[13].

Strict aseptic techniques were maintained throughout specimen collection, culture processing, and susceptibility testing to prevent contamination. Culture media were prepared and tested for sterility and performance according to manufacturer instructions. Standard control strains, including *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, were used to ensure quality control during culture and antimicrobial susceptibility testing[14].

3. Results

A total of 10 *Klebsiella* species were isolated from urine samples. They were studied for antibiotic sensitivity. Samples were collected from 8 females and 2 males of all ages. Antibiotic susceptibility of isolated *Klebsiella* was done. The above figure 1 shows that most participants in this study were female (80%) as compared to male (20%).

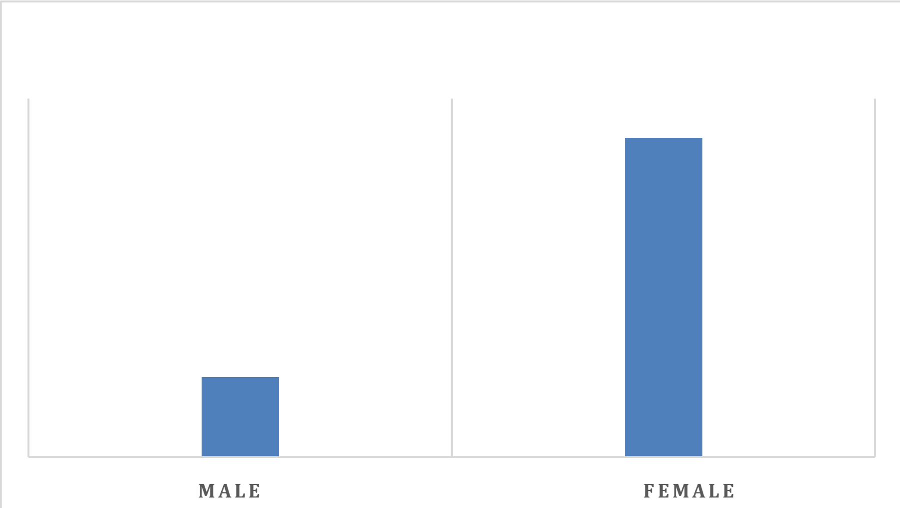

Figure 1. Gender Distribution

Table 1 shows the distribution of respondents according to age and gender (n=10). The majority of patients (40%) were in the 41–60 years age group, and all of them were female (40%). This was followed by patients aged 61 years and above (30%), where males accounted for 20% and females for 10%. Overall, most participants were female (80%), while males comprised 20% of the total study population.

Table 1. Distribution of the respondents according to Gender and Age **(n=10)**

Age	Male (%)	Female (%)	Total (%)
<20	0 (0%)	2 (20%)	2 (20%)
21-40	0 (0%)	1 (10%)	1 (10%)
41-60	0 (0%)	4 (40%)	4 (40%)
61 and more	2 (20%)	1 (10%)	3 (30%)
Total	2 (20%)	8 (80%)	10 (100%)

Table 2 presents the frequency of bacterial isolates among the study population (n=12). Out of 12 urine samples, 10 samples (83%) showed bacterial growth, while 2 samples (17%) showed no growth. This indicates a high proportion of culture-positive cases among the suspected UTI patients.

Table 2. Frequency of isolates in study population

Culture	Frequency	Percentage
Growth	10	83%
No Growth	2	17%
Total	12	100%

Table 3 shows the distribution of *Klebsiella* species among patients from different departments (n=10). The majority of isolates were obtained from OPD patients (70%), while 30% were from IPD patients. This indicates that *Klebsiella* infections were more frequently detected among outpatient cases in this study.

Table 3. Distribution of *Klebsiella* species among patients of IPD/OPD

Department	No. of isolates (n=10)	Percentage (%)
IPD	3	30%
OPD	7	70%
Total	10	100%

Table 4 shows the antibiotic sensitivity pattern of *Klebsiella* species isolated (n=10). The highest sensitivity was observed for Meropenem and Tigecycline (90% each), followed by Gentamicin (70%). Moderate sensitivity (50%) was found for Cefotaxime, Amoxycillin, Ceftazidime, Cefoxitin, Ampicillin, and Ciprofloxacin. Lower sensitivity was observed for Colistin (40%), while Doxycycline showed the least sensitivity (10%). This indicates that carbapenems and tigecycline were the most effective antibiotics against the isolated strains in this study.

Table 4. Sensitivity of *Klebsiella sp.* isolated (n=10)

Antibiotic	Sensitivity (%)
Cefotaxime	5 (50%)
Amoxycillin	5 (50%)
Ceftazidime	5 (50%)
Cefoxitin	5 (50%)
Meropenem	9 (90%)
Tigecycline	9 (90%)
Doxycycline	1 (10%)
Colistin	4 (40%)
Ampicillin	5 (50%)
Gentamycin	7 (70%)
Ciprofloxacin	5 (50%)

Table 5 shows the distribution of *Klebsiella pneumoniae* strains according to the number of antibiotics to which they were resistant. Two strains were resistant to seven antibiotics, and two strains were resistant to six antibiotics, indicating a high level of multidrug resistance. One strain each was resistant to five, four, three, and two antibiotics, while two strains showed resistance to only one antibiotic. Overall, the findings suggest that a considerable proportion of isolates exhibited resistance to multiple antibiotics.

Table 5. Number of antibiotics resistant to *Klebsiella pneumonia*

Serial No.	No. of Antibiotics Resistant to ***Klebsiella pneumoniae***	Number of Strains
1	7	2
2	6	2

Serial No.	No. of Antibiotics Resistant to *Klebsiella pneumoniae*	Number of Strains
3	5	1
4	4	1
5	3	1
6	2	1
7	1	2

Table 5. (*Continued*)

Table 6 shows the proportion of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) *Klebsiella pneumoniae* isolates. Out of the total isolates, 7 (70%) were identified as MDR, while no isolates were classified as XDR or PDR. This indicates a high prevalence of multidrug resistance among the study isolates, although extensive and pan drug resistance were not observed.

Table 6. Proportion of MDR, XDR, PDR

Serial No.	MDR	XDR	PDR
1	7(70%)	0	0

4. Discussion

Multidrug-resistant *Klebsiella pneumoniae* has emerged as a major global public health concern, particularly in hospital settings where inappropriate and excessive use of antibiotics accelerates the development of resistance. The increasing prevalence of resistant strains significantly limits therapeutic options and complicates the management of urinary tract infections (UTIs). The present study evaluated the isolation pattern and antibiotic susceptibility profile of *Klebsiella pneumoniae* from urine samples of patients with suspected UTI and provides insight into the current resistance trends in a tertiary care setting.

In this study, 10 *Klebsiella* isolates were obtained from 12 urine samples, with a culture positivity rate of 83%, indicating a high burden of bacterial UTI among clinically suspected cases. The majority of isolates were recovered from female patients (80%), which is consistent with the well-established higher incidence of UTIs in females due to anatomical and physiological factors such as a shorter urethra and its proximity to the perineal region. Although some previous studies have reported a higher proportion of infections among males, particularly in hospitalized or catheterized patients, the predominance of female patients in this study aligns with the general epidemiological pattern of UTIs[15].

Age-wise distribution showed that most patients (40%) belonged to the 41–60 years age group, followed by patients aged 61 years and above (30%). This finding suggests that middle-aged and elderly individuals are more vulnerable to *Klebsiella* UTIs, possibly due to declining immunity, presence of comorbidities such as diabetes mellitus, and increased healthcare exposure. Similar trends have been observed in other studies where *K. pneumoniae* infections were more common among adults and elderly populations[16].

Regarding the source of isolates, 70% of *Klebsiella* strains were recovered from OPD patients, while 30% were from IPD patients. This indicates that *Klebsiella pneumoniae* is not only a nosocomial pathogen but also a significant cause of community-acquired UTIs. The higher proportion in OPD patients may reflect widespread antibiotic misuse in the community, contributing to the selection of resistant strains outside hospital settings[17].

The antibiotic susceptibility pattern revealed that Meropenem and Tigecycline were the most effective agents, with 90% sensitivity, followed by Gentamicin (70%). Moderate sensitivity (50%) was observed for Cefotaxime, Amoxicillin, Ceftazidime, Cefoxitin, Ampicillin, and Ciprofloxacin. The lowest sensitivity was noted for Doxycycline (10%) and Colistin (40%). The high sensitivity to carbapenems indicates that these agents remain reliable treatment options for severe *Klebsiella* infections in this setting. However, the reduced sensitivity to commonly used antibiotics such as cephalosporins, fluoroquinolones, and penicillins suggests widespread resistance, possibly due to the production of extended-spectrum beta-lactamases (ESBLs) and other resistance mechanisms[18].

The resistance pattern further demonstrated that a considerable proportion of isolates were resistant to multiple antibiotics. Two strains were resistant to seven antibiotics and two strains to six antibiotics, indicating significant multidrug resistance. Overall, 70% of isolates were classified as multidrug-resistant (MDR), while no extensively drug-resistant (XDR) or pan drug-resistant (PDR) strains were detected. Although the absence of XDR and PDR strains is encouraging, the high prevalence of MDR isolates is alarming and highlights the urgent need for strict antibiotic stewardship and infection control measures[19].

Comparison with previous findings shows variation in sensitivity patterns, which may be attributed to geographical differences, sample size, prescribing practices, and infection control policies. Nevertheless, the overall trend of increasing multidrug resistance among *Klebsiella pneumoniae* isolates is consistent with global observations[20].

The findings of this study underscore the importance of routine culture and antibiotic susceptibility testing before initiating therapy[21]. Empirical treatment without laboratory guidance may lead to treatment failure and further emergence of resistant strains. Continuous surveillance, rational antibiotic use, and implementation of antimicrobial stewardship programs are essential to control the spread of multidrug-resistant *Klebsiella pneumoniae* and to improve patient outcomes.

5. Conclusion

Klebsiella pneumoniae remains a significant cause of urinary tract infections, particularly among middle-aged and elderly females, with a high proportion of multidrug-resistant strains. Carbapenems and tigecycline showed the highest efficacy, whereas resistance to commonly used antibiotics was considerable. These findings emphasize the need for regular monitoring of resistance patterns and judicious use of antibiotics to prevent further escalation of antimicrobial resistance.

Conflict of interest

The authors declare no conflict of interest

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