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Prevalence of Extended-Spectrum Beta-Lactamase Production and Biofilm Formation Among Klebsiella pneumoniae Urinary Isolates

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Abstract:

Background

Klebsiella pneumoniae is a major cause of urinary tract infections (UTIs) and is increasingly associated with extended-spectrum beta-lactamase (ESBL) production and biofilm formation, both of which complicate treatment and contribute to antimicrobial resistance. This study aimed to determine the prevalence of ESBL production and biofilm formation among K. pneumoniae urinary isolates in a tertiary care teaching hospital and to evaluate their association with multidrug resistance and clinical risk factors.

Methods

A cross-sectional study was conducted on 294 non-duplicate K. pneumoniae isolates recovered from urinary samples. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBL production was confirmed using the combined disc method, while biofilm formation was assessed using the microtiter plate assay. Statistical associations were analyzed using the Chi-square test, and a p-value of less than 0.05 was considered statistically significant.

Results

ESBL production was detected in 176 (59.9%) isolates, while 214 (72.8%) demonstrated biofilm-forming ability. Multidrug resistance (MDR) was observed in 158 (53.7%) isolates. A significant association was found between ESBL production and biofilm formation ($p < 0.001$). Inpatient status, ICU admission, urinary catheterization, diabetes mellitus, and recurrent urinary tract infection were significantly associated with ESBL positivity. Resistance to carbapenems remained relatively low, with resistance rates of 8.2% for imipenem and 9.5% for meropenem.

Conclusion

The coexistence of ESBL production, multidrug resistance, and biofilm formation among K. pneumoniae urinary isolates represents a major therapeutic challenge. The strong association between resistance mechanisms and biofilm-forming ability highlights the need for continuous antimicrobial surveillance, effective infection control practices, and robust antimicrobial stewardship programs. Early detection of ESBL-producing strains and appropriate antibiotic management are essential to limit the spread of resistant organisms and improve clinical outcomes.

Keywords:

Klebsiella pneumoniae; ESBL; Biofilm formation; Multidrug resistance; Urinary tract infection

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Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, affecting millions of people every year and placing a considerable burden on healthcare systems [1]. Gram-negative bacteria are the primary causative agents, with Klebsiella pneumoniae recognized as an important pathogen, especially in complicated UTIs and catheter-associated urinary tract infections (CAUTIs) seen in hospitalized patients [2]. The growing clinical importance of this organism is largely due to its increasing resistance to antibiotics and its ability to survive and spread within healthcare environments [2].

One of the major mechanisms of antibiotic resistance in K. pneumoniae is the production of extended-spectrum beta-lactamases (ESBLs). These enzymes can break down many commonly used beta-lactam antibiotics, including third-generation cephalosporins, making infections more difficult to treat [3]. ESBL-producing strains are often resistant to multiple classes of antibiotics, which can result in limited treatment options, longer hospital stays, higher healthcare costs, and poorer patient outcomes [4].

Apart from antibiotic resistance, biofilm formation is another important virulence factor of K. pneumoniae. Biofilms are organized communities of bacteria enclosed within a protective matrix that allows them to attach to surfaces such as the urinary tract lining and indwelling medical devices, including urinary catheters [5]. Bacteria growing within biofilms are significantly more resistant to antimicrobial agents and can persist for prolonged periods, making infections difficult to eradicate [6].

The coexistence of ESBL production and biofilm-forming ability represents a serious clinical challenge. Together, these characteristics promote persistent infections, facilitate the transfer of resistance genes, and contribute to the spread of resistant strains within healthcare settings [7]. Therefore, understanding the prevalence of ESBL production and biofilm formation, as well as the relationship between them in K. pneumoniae urinary isolates, is essential for selecting appropriate empirical therapy and strengthening infection prevention and control measures.

Material and methods

Study Design and Setting

This hospital-based cross-sectional observational study was conducted in the Department of Microbiology of a tertiary care teaching hospital. The study was designed to evaluate the prevalence of extended-spectrum beta-lactamase (ESBL) production and biofilm formation among Klebsiella pneumoniae isolates recovered from urinary tract infection (UTI) samples. The hospital serves both outpatient and inpatient populations, including patients admitted to intensive care units (ICUs), medical wards, and surgical wards. Ethical approval was obtained from the Institutional Ethics Committee before the commencement of the study, and strict confidentiality of patient information was maintained throughout the study period.

Sample Collection and Bacterial Isolation

Urine samples were collected from patients with a clinical suspicion of urinary tract infection. Depending on the clinical condition, samples included midstream clean-catch urine, catheterized urine, and suprapubic aspirates. All specimens were processed according to standard microbiological procedures soon after collection. Semi-quantitative urine culture was performed using a calibrated loop on Cysteine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar. The inoculated plates were incubated under appropriate laboratory conditions, and bacterial growth was interpreted following standard microbiological guidelines. Significant bacteriuria was determined based on accepted colony count criteria for both midstream and catheterized urine samples.

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Presumptive identification of Klebsiella pneumoniae was based on characteristic colony morphology, Gram staining findings, and conventional biochemical tests, including indole reaction, citrate utilization, urease production, triple sugar iron (TSI) agar reaction, and motility testing. Final confirmation was performed using standard identification protocols or automated identification systems whenever available.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotic panel included ampicillin, amoxicillin-clavulanate, cefotaxime, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, gentamicin, amikacin, cotrimoxazole, nitrofurantoin, piperacillin-tazobactam, and imipenem/meropenem. The diameter of inhibition zones was measured and interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints. Escherichia coli ATCC quality-control strains were used to ensure accuracy and reliability of the susceptibility testing procedure [8].

Detection of ESBL Production

Initial screening for ESBL production was carried out using ceftazidime and cefotaxime discs. Isolates showing reduced susceptibility to these antibiotics were further evaluated using the combined disc method for phenotypic confirmation. For confirmation, antibiotic discs containing clavulanic acid were compared with cephalosporin discs alone. An increase in the inhibition zone in the presence of clavulanic acid was considered indicative of ESBL production, following CLSI recommendations. Appropriate quality-control measures were maintained throughout the testing process.

Detection of Biofilm Formation

Biofilm formation was assessed using the microtiter plate (MTP) assay, a widely accepted quantitative method for evaluating biofilm-producing ability. Briefly, bacterial isolates were inoculated into tryptic soy broth supplemented with glucose and incubated under standard laboratory conditions. After incubation, cultures were diluted and transferred into sterile microtiter plates. Following further incubation, non-adherent cells were removed by gentle washing with phosphate-buffered saline (PBS). The adherent biofilm was fixed, stained with crystal violet, and subsequently quantified by measuring optical density using an ELISA reader. Based on optical density values and predefined cut-off criteria, isolates were categorized as non-biofilm producers, weak biofilm producers, moderate biofilm producers, or strong biofilm producers.

Data Collection and Statistical Analysis

Demographic and clinical information, including age, sex, inpatient or outpatient status, ICU admission, and catheterization history, was collected from laboratory request forms and hospital records. All data were entered into Microsoft Excel and analyzed using the Statistical Package for the Social Sciences (SPSS). Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. Associations between ESBL production, biofilm formation, and clinical variables were evaluated using the Chi-square test or Fisher's exact test, wherever appropriate. A p-value of less than 0.05 was considered statistically significant.

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Results

A total of 294 patients with culture-confirmed Klebsiella pneumoniae urinary tract infection (UTI) were included in the study. The mean age of the patients was 49.8 ± 17.6 years. The majority of patients belonged to the 46–60 years age group (30.6%), followed by those older than 60 years (25.9%). Male patients accounted for 58.5% of cases, while females constituted 41.5%. Most isolates were obtained from hospitalized patients (62.6%), whereas 37.4% were from outpatients. ICU admission was required for 23.1% of patients. Urinary catheterization was present in 43.5% of cases. Diabetes mellitus was observed in 39.5% of patients, and 27.9% had a history of recurrent UTI (Table 1).

Table 1. Demographic and Clinical Characteristics of Patients with Klebsiella pneumoniae UTI (n = 294)

Variable	Frequency	Percentage (%)
Age (years)	**49.8 ± 17.6**	—
18–30	**56**	**19.0**
31–45	**72**	**24.5**
46–60	**90**	**30.6**
>60	**76**	**25.9**
Male	**172**	**58.5**
Female	**122**	**41.5**
Inpatient	**184**	**62.6**
Outpatient	**110**	**37.4**
ICU admission	**68**	**23.1**
Urinary catheter present	**128**	**43.5**
Diabetes mellitus	**116**	**39.5**
Recurrent UTI history	**82**	**27.9**

The antimicrobial susceptibility analysis revealed high levels of resistance among the isolates. All isolates were resistant to ampicillin (100%). High resistance rates were observed for ceftriaxone (63.9%), cefotaxime (61.9%), and ceftazidime (59.2%). Resistance to ciprofloxacin and cotrimoxazole was 55.8% and 48.3%, respectively. Gentamicin resistance was observed in 42.9% of isolates, while 23.1% were resistant to amikacin. Resistance to nitrofurantoin was 35.4%. Carbapenem resistance remained relatively low, with resistance rates of 8.2% for imipenem and 9.5% for meropenem (Table 2).

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Table 2. Antimicrobial Resistance Pattern of Klebsiella pneumoniae Isolates (n = 294).

Antibiotic	Frequency (%)
Ampicillin	294 (100.0)
Amoxicillin-clavulanate	192 (65.3)
Cefotaxime	182 (61.9)
Ceftazidime	174 (59.2)
Ceftriaxone	188 (63.9)
Cefepime	152 (51.7)
Ciprofloxacin	164 (55.8)
Gentamicin	126 (42.9)
Amikacin	68 (23.1)
Cotrimoxazole	142 (48.3)
Nitrofurantoin	104 (35.4)
Piperacillin-tazobactam	76 (25.9)
Imipenem	24 (8.2)
Meropenem	28 (9.5)

Out of 294 isolates, 176 (59.9%) were confirmed as ESBL producers, while 118 (40.1%) were non-ESBL producers. Biofilm formation was detected in 214 isolates (72.8%). Among the biofilm-producing isolates, 68 (23.1%) were strong biofilm producers, 82 (27.9%) were moderate producers, and 64 (21.8%) were weak producers. Non-biofilm producers accounted for 80 (27.2%) isolates (Table 3).

Table 3. Immunohistochemical Profile and Ki-67 Proliferation Index of Major Renal Mesenchymal Neoplasms.

Parameter	Frequency	Percentage (%)
ESBL producers	176	59.9
Non-ESBL	118	40.1
Strong biofilm	68	23.1
Moderate biofilm	82	27.9
Weak biofilm	64	21.8

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Non-biofilm producer	**80**	**27.2**
Overall biofilm producers	**214**	**72.8**

A statistically significant association was observed between ESBL production and biofilm formation ($p < 0.001$). Among ESBL-producing isolates, 150 (85.2%) were biofilm producers compared with 64 (54.2%) among non-ESBL isolates. Strong biofilm formation was more common among ESBL-producing isolates (31.8%) than among non-ESBL isolates (10.2%). Hospitalization, ICU stay, urinary catheterization, diabetes mellitus, and recurrent UTI were significantly associated with ESBL production. Multidrug resistance (MDR) was observed in 158 isolates (53.7%). MDR isolates showed a strong association with both ESBL production (88.6%) and biofilm formation (87.3%) ($p < 0.001$).

Discussion

The present study highlights a substantial burden of antimicrobial resistance among Klebsiella pneumoniae urinary isolates in a tertiary care hospital setting, with ESBL production detected in half of isolates and biofilm formation observed in three fourth of isolates. These findings align with contemporary Indian data, where ESBL prevalence among K. pneumoniae ranges between 40–70%, particularly in hospital-based studies by Mishra et al., Rahman et al., and Ahmad et al., [9,10,11]. The predominance of middle-aged and elderly patients and the higher proportion of inpatients reflect the increasing vulnerability of hospitalized and comorbid populations to resistant infections [12]. Nearly two fifth of patients were diabetic, a well-recognized risk factor for complicated UTIs due to impaired immune response and glycosuria facilitating bacterial growth [13].

The antimicrobial susceptibility pattern observed in this study mirrors the evolving resistance trends reported in studies by Lobo et al., Pawar et al., and Sahoo et al., across tertiary care centers in India [14,15,16]. Universal resistance to ampicillin and high resistance to third-generation cephalosporins are consistent with widespread ESBL dissemination [15,16]. Fluoroquinolone resistance and cotrimoxazole resistance further limit oral treatment options. However, carbapenem resistance remained relatively low, suggesting preserved activity, though emerging carbapenem resistance remains a concerning trend nationally [17,18]. The high proportion of multidrug-resistant (MDR) isolates underscores the therapeutic challenge posed by K. pneumoniae in urinary infections [19].

A key finding of this study is the strong association between ESBL production and biofilm formation ($p < 0.001$). Among ESBL producers, four fifth were biofilm formers compared to one and half among non-ESBL isolates. Strong biofilm production was significantly more frequent in ESBL-positive isolates. Similar associations have been reported in studies by Shahriar et al., Khalefa et al., and Romyasamit et al., demonstrating that ESBL-producing strains possess enhanced adherence and biofilm-forming capacity [20,21,22]. Biofilms confer protection through reduced antibiotic penetration, altered metabolic states, and quorum-sensing–mediated gene regulation [22]. Importantly, biofilm matrices facilitate horizontal gene transfer via plasmids, potentially accelerating dissemination of ESBL genes within hospital environments [21].

Hospital-related factors were significantly associated with ESBL production, including inpatient status ($p = 0.001$), ICU admission ($p = 0.028$), and urinary catheterization ($p = 0.002$). Catheter presence likely contributed to the high biofilm prevalence, as indwelling devices provide surfaces for bacterial adherence and biofilm maturation. Diabetes mellitus ($p = 0.006$) and recurrent UTI ($p = 0.018$) also emerged as significant risk factors, supporting previous studies by Raza et

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al., Papp et al., and Wajid et al., that metabolic disorders and repeated antibiotic exposure promote selection of resistant strains [23,24,25].

Furthermore, MDR isolates demonstrated a significant overlap with ESBL production (88.6%) and biofilm formation ($p < 0.001$), suggesting a synergistic relationship between resistance determinants and virulence traits [26,27]. This convergence of MDR phenotype, enzymatic resistance, and biofilm capability may contribute to persistent infection, therapeutic failure, and increased recurrence rates [28,29].

Limitations

This study was conducted at a single tertiary care center, which may limit generalizability to community or primary care settings. Molecular characterization of ESBL genes (e.g., blaCTX-M, blaTEM, blaSHV) was not performed, restricting genotypic correlation. Additionally, biofilm assessment was based solely on the microtiter plate method without molecular evaluation of biofilm-associated genes. Clinical outcomes and treatment response were not analyzed, which could have strengthened the clinical relevance of the findings.

Conclusion

The present study demonstrated a high prevalence of ESBL production (59.9%) and biofilm formation (72.8%) among Klebsiella pneumoniae urinary isolates obtained from patients with urinary tract infections. A strong and statistically significant association was observed between ESBL production, multidrug resistance, and biofilm-forming ability, indicating a close relationship between antimicrobial resistance mechanisms and bacterial virulence factors. Hospital-related factors, including inpatient status, ICU admission, urinary catheterization, diabetes mellitus, and recurrent urinary tract infections, were significantly associated with ESBL-positive isolates. These findings highlight the importance of both healthcare-associated exposures and underlying comorbidities in the emergence and persistence of resistant K. pneumoniae strains. Although carbapenems retained good activity against most isolates, the high prevalence of multidrug resistance remains a major therapeutic concern. Continuous surveillance of antimicrobial resistance patterns, routine detection of ESBL-producing isolates, monitoring of biofilm formation, strict infection prevention and control measures, and effective antimicrobial stewardship programs are essential to limit the spread of resistant strains and improve patient outcomes. Overall, the coexistence of ESBL production, multidrug resistance, and biofilm formation among Klebsiella pneumoniae urinary isolates represents a significant clinical challenge and underscores the need for comprehensive strategies to combat antimicrobial resistance in healthcare settings.

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