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Original Research Article

COEXISTENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCTION AND BIOFILM FORMATION IN KLEBSIELLA PNEUMONIA CAUSING URINARY TRACT INFECTIONS

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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 promote antimicrobial resistance. This study aimed to determine the prevalence of ESBL production and biofilm formation among K. pneumoniae urinary isolates in a tertiary care hospital and to evaluate their association with multidrug resistance and clinical risk factors.

Methods: A cross-sectional study was conducted on 147 non-duplicate K. pneumoniae isolates obtained from urinary samples. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method as per CLSI guidelines. ESBL production was confirmed by the combined disc method. Biofilm formation was assessed using the microtiter plate assay. Associations were analyzed using the Chi-square test, with $p < 0.05$ considered statistically significant.

Results: ESBL production was detected in 59.9% of isolates, while 72.8% demonstrated biofilm-forming ability. Multidrug resistance was observed in 53.7% of isolates. A significant association was found between ESBL production and biofilm formation ($p < 0.001$). Inpatient status, ICU stay, urinary catheterization, diabetes mellitus, and recurrent UTI were significantly associated with ESBL positivity. Carbapenem resistance remained relatively low (8–9%).

Conclusion: The high coexistence of ESBL production, multidrug resistance, and biofilm formation among K. pneumoniae urinary isolates poses a significant therapeutic challenge. Strengthened surveillance, infection control measures, and rational antibiotic stewardship are essential to mitigate the spread of resistant strains in tertiary care settings.

Keywords: Klebsiella pneumoniae; ESBL; Biofilm formation; Multidrug resistance; Urinary tract infection.

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections globally, accounting for nearly 150–200 million cases annually and contributing substantially to healthcare burden [1]. Gram-negative bacilli predominate, with

Klebsiella pneumoniae emerging as a major pathogen, particularly in complicated and catheter-associated UTIs (CAUTI) in tertiary care hospitals [2]. Its clinical importance has increased due to rising antimicrobial resistance and its ability to persist in hospital environments [2].

One of the most significant resistance mechanisms in K. pneumoniae is the production of extended-spectrum beta-lactamases (ESBLs). These plasmid-mediated enzymes hydrolyze third-generation cephalosporins and monobactams, limiting the efficacy of commonly used antibiotics [3]. Globally, ESBL prevalence among Enterobacterales ranges from 20% to over 60%, with studies from India reporting rates of 40–70% in urinary isolates of K. pneumoniae [4]. ESBL-producing strains are frequently multidrug-resistant, often necessitating carbapenem therapy and leading to prolonged hospitalization, increased costs, and adverse clinical outcomes [5].

In addition to enzymatic resistance, biofilm formation is a key virulence factor. Biofilms are structured bacterial communities embedded in an extracellular matrix that adhere to uroepithelium and indwelling devices such as urinary catheters. Up to 65–80% of device-associated infections involve biofilm-forming organisms [6]. Bacteria within biofilms exhibit markedly reduced antimicrobial susceptibility and enhanced persistence [6].

The coexistence of ESBL production and biofilm-forming ability poses a dual threat, facilitating chronic infection, horizontal gene transfer, and dissemination within healthcare settings [7]. Therefore, evaluating the prevalence and association of ESBL production and biofilm formation among K. pneumoniae urinary isolates in tertiary care hospitals is crucial for guiding empirical therapy and strengthening infection control strategies.

Materials 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 over a period of 18 months (from June 2023 to December 2024). The study aimed to determine the prevalence of extended-spectrum beta-lactamase (ESBL) production and biofilm formation among Klebsiella pneumoniae isolates obtained from urinary tract samples. The hospital caters to both outpatient and inpatient populations, including intensive care units (ICUs), medical, and surgical wards. Ethical clearance was obtained from the Institutional Ethics Committee prior to study initiation, and patient confidentiality was strictly maintained.

Sample Collection and Bacterial Isolation: Urine samples were collected from patients clinically suspected of urinary tract infection (UTI), including midstream clean-catch urine, catheterized urine, and suprapubic aspirates where indicated. Samples were processed within 2 hours of collection. Semi-quantitative culture was performed using a calibrated loop (0.001 mL) on Cysteine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar plates, followed by incubation at 37°C for 18–24 hours. Significant bacteriuria was defined as colony counts $\geq 10^5$ CFU/mL for midstream urine and $\geq 10^4$ CFU/mL for catheterized samples, interpreted according to standard microbiological guidelines.

Presumptive identification of Klebsiella pneumoniae was based on colony morphology (large, mucoid, lactose-fermenting colonies on MacConkey agar), Gram staining (Gram-negative bacilli), and standard biochemical tests including indole (negative), citrate utilization (positive), urease production (positive), triple sugar iron (TSI) agar reaction (acid/acid with gas, no H_2S), and motility test (non-motile). Confirmation was performed using standard biochemical identification

protocols or automated systems where available.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing (AST) was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. Antibiotics tested included ampicillin, amoxicillin-clavulanate, cefotaxime, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, gentamicin, amikacin, cotrimoxazole, nitrofurantoin, piperacillin-tazobactam, and imipenem/meropenem. Zone diameters were measured and interpreted as sensitive, intermediate, or resistant based on CLSI breakpoints [8]. Escherichia coli ATCC 25922 was used as the quality control strain.

Detection of ESBL Production: Screening for ESBL production was initially performed using ceftazidime (30 µg) and cefotaxime (30 µg) discs. Isolates showing reduced susceptibility (zone diameter ≤ CLSI screening cut-offs) were subjected to phenotypic confirmation using the combined disc method. In this method, ceftazidime (30 µg) alone and ceftazidime-clavulanic acid (30/10 µg), as well as cefotaxime (30 µg) alone and cefotaxime-clavulanic acid (30/10 µg), were placed 25 mm apart on Mueller–Hinton agar inoculated with a 0.5 McFarland standardized bacterial suspension. An increase of ≥5 mm in zone diameter in the presence of clavulanic acid compared to the cephalosporin alone was interpreted as confirmation of ESBL production, as per CLSI recommendations.

Detection of Biofilm Formation: Biofilm formation was assessed using the microtiter plate (MTP) assay, which is a quantitative method for detecting biofilm production. Briefly, isolates were inoculated into tryptic soy broth (TSB) supplemented with 1% glucose and incubated at 37°C for 24 hours. The culture was diluted (1:100), and 200 µL was added to sterile 96-well flat-bottom polystyrene microtiter plates in triplicate. After incubation at 37°C for 24 hours, wells were gently washed three times with phosphate-buffered saline (PBS) to remove planktonic cells and allowed to dry. Adherent cells were fixed with methanol, stained with 0.1% crystal violet for 15 minutes, and excess stain was rinsed off with distilled water. The bound dye was solubilized using 95% ethanol, and optical density (OD) was measured at 570 nm using an ELISA reader. Isolates were categorized as non-biofilm producers, weak, moderate, or strong biofilm producers based on optical density cut-off (ODc) values calculated from negative controls.

Data Collection and Statistical Analysis: Demographic and clinical details including age, sex, inpatient/outpatient status, and catheterization history were collected from laboratory requisition forms and hospital records. Data were entered into Microsoft Excel and analyzed using SPSS version 20.0. Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. The association between ESBL production and biofilm formation was analyzed using the Chi-square test or Fisher's exact test where appropriate. A p-value <0.05 was considered statistically significant.

Results

Among the 147 patients with culture-confirmed Klebsiella pneumoniae UTI, the mean age was 49.8 ± 17.6 years, with the majority belonging to the 46–60 years age group (30.6%), followed by those above 60 years (25.9%). Males constituted 58.5% of cases.

A higher proportion of isolates were obtained from inpatients (62.6%) compared to outpatients (37.4%), with 23.1% requiring ICU admission. Urinary catheterization was present in 43.5% of patients. Diabetes mellitus was noted in 39.5%, and 27.9% had a history of recurrent UTI (Table 1).

Table 1: Demographic and Clinical Characteristics of Patients with Klebsiella pneumoniae Urinary Tract Infection (n = 147)

Variable	Frequency/mean ± SD	Percentage (%)
Age (years)	49.8 ± 17.6	—
Age Group		
18–30	28	19
31–45	36	24.5
46–60	45	30.6
>60	38	25.9
Sex		
Male	86	58.5
Female	61	41.5
Patient		
Inpatient	92	62.6
Outpatient	55	37.4
ICU admission	34	23.1
Urinary catheter present	64	43.5
Diabetes mellitus	58	39.5
Recurrent UTI history	41	27.9

(ICU: Intensive Care Unit; UTI: Urinary Tract Infection)

All isolates were resistant to ampicillin (100%). High resistance rates were observed to third-generation cephalosporins—ceftriaxone (63.9%), cefotaxime (61.9%), and ceftazidime (59.2%). Resistance to ciprofloxacin was 55.8%, while cotrimoxazole resistance was 48.3%. Aminoglycoside resistance was moderate, with 42.9% resistant to gentamicin and 23.1% to amikacin. Resistance to nitrofurantoin was 35.4%. Notably, carbapenem resistance remained low, with 8.2% and 9.5% resistance to imipenem and meropenem respectively (Table 2).

Table 2: Antimicrobial Resistance Pattern of Klebsiella pneumoniae Isolates (n = 147)

Antibiotic	Frequency (%)
Ampicillin	147 (100)
Amoxicillin-clavulanate	96 (65.3)
Cefotaxime	91 (61.9)
Ceftazidime	87 (59.2)
Ceftriaxone	94 (63.9)
Cefepime	76 (51.7)
Ciprofloxacin	82 (55.8)
Gentamicin	63 (42.9)
Amikacin	34 (23.1)
Cotrimoxazole	71 (48.3)
Nitrofurantoin	52 (35.4)
Piperacillin-tazobactam	38 (25.9)
Imipenem	12 (8.2)
Meropenem	14 (9.5)

(AST performed as per CLSI guidelines.)

Out of 147 isolates, 88 (59.9%) were confirmed as ESBL producers. Biofilm formation was detected in 107 isolates (72.8%). Among these, 23.1% were strong biofilm producers, 27.9% moderate producers, and 21.8% weak producers, while 27.2% were non-biofilm producers. This demonstrates a high prevalence of both ESBL production and biofilm-forming capability among urinary isolates (Table 3).

Table 3: Prevalence of ESBL Production and Biofilm Formation Among Klebsiella pneumoniae Isolates (n = 147)

Parameter	Frequency	Percentage (%)
ESBL producers	88	59.9
Non-ESBL	59	40.1
Biofilm formation		
Strong	34	23.1
Moderate	41	27.9
Weak	32	21.8
Non-biofilm producer	40	27.2
Overall biofilm producers (weak+moderate+strong)	107	72.8

(ESBL: Extended-Spectrum Beta-Lactamase; Biofilm detection by microtiter plate method.)

A significant association was observed between ESBL production and biofilm formation ($p < 0.001$). Among ESBL-producing isolates, 85.2% were biofilm producers compared to 54.2% among non-ESBL isolates. Strong biofilm formation was more frequent in ESBL producers (31.8%) than non-ESBL isolates (10.2%). Conversely, non-biofilm production was significantly higher in non-ESBL isolates (45.8%) compared to ESBL producers (14.8%) (Table 4).

Table 4: Association Between ESBL Production and Biofilm Formation in Klebsiella pneumoniae (n = 147)

Biofilm Category	ESBL (n=88)	Non-ESBL (n=59)	p-value
	Frequency (%)		
Strong	28 (31.8)	6 (10.2)	<0.001
Moderate	32 (36.4)	9 (15.3)	
Weak	15 (17.0)	17 (28.8)	
Non-biofilm	13 (14.8)	27 (45.8)	
Overall biofilm positive	75 (85.2)	32 (54.2)	

Inpatient status was significantly associated with ESBL production (73.9% vs 45.8%; $p = 0.001$). ICU admission showed a significant association (29.5% vs 13.6%; $p = 0.028$). Urinary catheterization was more common among ESBL-positive cases (54.5%) compared to non-ESBL cases (27.1%) ($p = 0.002$). Diabetes mellitus (48.9% vs 25.4%; $p = 0.006$) and history of recurrent UTI (35.2% vs 16.9%; $p = 0.018$) were also significantly associated with ESBL production (Table 5).

Table 5: Risk Factors Associated with ESBL Production Among Klebsiella pneumoniae Isolates (n = 147)

Risk Factor	ESBL (n=88)	Non-ESBL (n=59)	p-value
	Frequency (%)		
Inpatient status	65 (73.9)	27 (45.8)	0.001
ICU stay	26 (29.5)	8 (13.6)	0.028
Urinary catheter	48 (54.5)	16 (27.1)	0.002
Diabetes mellitus	43 (48.9)	15 (25.4)	0.006
Recurrent UTI	31 (35.2)	10 (16.9)	0.018

Multidrug resistance was observed in 79 isolates (53.7%). MDR isolates showed a strong association with ESBL production (88.6% vs 26.5%; $p < 0.001$). Similarly, biofilm formation was significantly more common among MDR isolates (87.3%) compared to non-MDR isolates (55.9%) ($p < 0.001$) (Table 6).

Table 6: Correlation of Multidrug Resistance (MDR) with ESBL Production and Biofilm Formation (n = 147)

Parameter	MDR Present (n=79)	MDR Absent (n=68)	p-value
	Frequency (%)		
Biofilm positive	69 (87.3)	38 (55.9)	<0.001
ESBL positive	70 (88.6)	18 (26.5)	<0.001

(MDR defined as resistance to ≥3 antimicrobial classes.)

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 59.9% of isolates and biofilm formation observed in 72.8%. 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 (mean age 49.8 ± 17.6 years) and the higher proportion of inpatients (62.6%) reflect the increasing vulnerability of hospitalized and comorbid populations to resistant infections [12]. Nearly 40% 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 (cefotaxime 61.9%, ceftriaxone 63.9%) are consistent with widespread ESBL dissemination [15,16]. Fluoroquinolone resistance (55.8%) and cotrimoxazole resistance (48.3%) further limit oral treatment options. However, carbapenem resistance remained relatively low (8–9%), suggesting preserved activity, though emerging carbapenem resistance remains a concerning trend nationally [17,18]. The high proportion of multidrug-resistant (MDR) isolates (53.7%) 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, 85.2% were biofilm formers compared to 54.2% among non-ESBL isolates. Strong biofilm production was significantly more frequent in ESBL-positive isolates (31.8% vs 10.2%). Similar associations have been reported in studies by Shahriar et al., Khalefaet al., and Romyasamitet 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 (43.5% overall) 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 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 (87.3%) ($p < 0.001$), 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 demonstrates a high prevalence of ESBL production (59.9%) and biofilm formation (72.8%) among Klebsiella pneumoniae urinary isolates in a tertiary care hospital. A strong and statistically significant association was observed between ESBL production, multidrug resistance, and biofilm-forming capacity, highlighting the convergence of resistance and virulence mechanisms. Hospital-related factors such as catheterization, ICU stay, diabetes mellitus, and recurrent UTI were significant risk determinants. Although carbapenems retained good activity, rising multidrug resistance underscores the need for continuous antimicrobial surveillance. Routine ESBL detection, monitoring of biofilm formation, stringent infection control practices, and antimicrobial stewardship programs are essential to curb the spread of resistant K. pneumoniae and improve therapeutic outcomes in urinary tract infections.

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