RESEARCH PAPER

Multidrug Resistant Klebsiella Pneumoniae in Hemodialysis Patients with Central Line Associated Blood Stream Infection in Minia University Hospital: Antimicrobial Resistance Profiles and Phenotypic Detection of Resistance Mechanisms

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Background

Klebsiella pneumoniae is an important global health problem because of its ability to develop multidrug resistance (MDR) and form biofilms. Biofilm formation can reduce antibiotic effectiveness and make bacterial infections more difficult to treat.

Objective

This study aimed to investigate the relationship between biofilm formation and major antimicrobial resistance phenotypes, including extended-spectrum beta-lactamase (ESBL), carbapenem resistance (CRE), AmpC.

Methods

Forty-three clinical *K. pneumoniae* isolates were evaluated for Biofilm formation and antimicrobial susceptibility patterns .

Results

Biofilm formation was detected in 38 of 43 isolates (88.4%) using the MTP assay. Among the biofilm-producing isolates, 39.5% showed strong biofilm formation, 39.5% moderate formation, and 9.3% weak formation. Biofilm-producing isolates had significantly higher rates of ESBL production than non-biofilm-producing isolates (86.8% vs. 20.0%, $p = 0.005$). MDR was significantly more common among biofilm-producing isolates (100% vs. 60.0%, $p = 0.0111$). AmpC production (68.4%, $p = 0.0556$) and CRE (73.7%, $p = 0.1529$) were also more frequent among biofilm-producing isolates, although these associations were not statistically significant.

Conclusion

Biofilm formation was strongly associated with ESBL production and MDRO status among clinical *K. pneumoniae* isolates. The high prevalence of both biofilm formation and antimicrobial resistance highlights the difficulty of treating these infections and supports the need for effective strategies to improve antimicrobial activity against biofilm-associated bacteria.

Keywords: *Klebsiella pneumoniae* , Biofilm Formation, Multidrug Resistance, Extended-Spectrum beta-Lactamase (ESBL) And MTP.
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INTRODUCTION

Klebsiella pneumoniae is an important opportunistic pathogen and a major cause of healthcare-associated infections, including pneumonia, urinary tract infections, bloodstream infections, and catheter-associated infections (Cabrera et al., 2025; Shah et al., 2025). Its clinical importance has increased because of the rapid emergence of multidrug-resistant (MDR) strains, particularly those producing extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases (Cabrera et al., 2025; Koli et al., 2025). Carbapenem-resistant *K. pneumoniae* is of particular concern because it markedly limits therapeutic options for severe infections (Shah et al., 2025; Koli et al., 2025).

In addition to antimicrobial resistance, biofilm formation is an important mechanism that contributes to the persistence of *K. pneumoniae* (Li et al., 2024). Biofilms consist of bacterial communities surrounded by an extracellular polymeric substance (EPS) matrix containing polysaccharides, proteins, and extracellular DNA, which facilitates bacterial attachment and persistence (Li et al., 2024). This structure can reduce antimicrobial penetration and activity, making biofilm-associated infections more difficult to treat (Khalefa et al., 2025).

Several studies have suggested an association between biofilm formation and antimicrobial resistance in *K. pneumoniae* (Li et al., 2024; Kumar et al., 2026). Resistant isolates, particularly ESBL-producing and carbapenem-resistant strains, may show a greater ability to form biofilms (Khalefa et al., 2025; Kumar et al., 2026). However, the strength of this association may vary according to the bacterial population, resistance phenotype, and methods used to assess biofilm formation, highlighting the need for further clinical investigation (Li et al., 2024; Kumar et al., 2026).

The Microtiter Plate (MTP) assay is widely used as a quantitative method for assessing biofilm biomass, while Congo Red Agar (CRA) provides a simple qualitative screening method (Khoshnood et al., 2023; Abdulqadir et al., 2025).

Therefore, this study aimed to evaluate biofilm formation among clinical *K. pneumoniae* isolates using MTP and CRA methods and to investigate its association with major resistance phenotypes, including ESBL, AmpC and CRE. The diagnostic performance of CRA was also compared with the MTP assay. These findings may improve understanding of the relationship between biofilm formation and antimicrobial resistance and may provide a microbiological basis for future strategies targeting biofilm-associated infections.

2. Materials and Methods

2.1. Study Cohort and Clinical Isolates

A total of 43 clinical *Klebsiella pneumoniae* isolates were recovered from hemodialysis patient diagnosed as CLABSI. Demographic and clinical data, including catheterization and dialysis durations, were recorded. Laboratory parameters, including C-reactive protein (CRP), total leukocyte count (TLC), serum creatinine, and random blood sugar (RBS), were also documented.

2.2. Phenotypic Biofilm Quantification

Microtiter Plate (MTP) Assay (Andersen et al., 2024)

Biofilm formation was quantitatively assessed using the microtiter plate method. The optical density (OD) was measured at 570 nm using a microplate reader..

Congo Red Agar (CRA) Assay: (Turu, et al ., 2025)
Biofilm production was qualitatively assessed using Congo Red Agar. Black crystalline colonies were considered positive for biofilm production, whereas red or pink colonies were considered negative.

2.3. Antimicrobial Susceptibility and High-Risk Resistance Phenotypes

Antimicrobial susceptibility testing was performed using the VITEK 2 AST-GN automated system. Minimum inhibitory concentrations (MICs) were interpreted according to the Clinical and Laboratory Standards Institute (CLSI ,2025) .

The isolates were also evaluated for major antimicrobial resistance phenotypes, including ESBL, CRE and AmpC β-lactamase .

2.4. Statistical Analysis

Categorical variables were presented as frequencies and percentages. Continuous variables were expressed

as mean ± standard deviation (SD) or median and interquartile range (IQR), as appropriate. Associations between biofilm production and antimicrobial resistance phenotypes were assessed using Fisher's exact test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength of associations. A two-sided p-value < 0.05 was considered statistically significant.

3. RESULTS

Table 1. Demographic and Clinical Characteristics of the Studied CLABSI Patients

Characteristic	Value
Age, mean ± SD (years)	49.93 ± 9.28
Diabetes mellitus, n (%)	25 (58.1)
Hypertension, n (%)	19 (44.2)
Fever, n (%)	40 (93.0)
Vomiting, n (%)	16 (37.2)
Previous/prophylactic antibiotic use, n (%)	28 (65.1)
Catheterization duration, mean ± SD (days)	11.95 ± 3.63
ICU stay, mean ± SD (days)	1.05 ± 1.45
Dialysis duration, mean ± SD	22.88 ± 10.05

Category variables are expressed as frequency (n) and percentage (%).

Continuous variables are expressed as Mean ± Standard Deviation (SD).

The mean age of the patients was 49.93 ± 9.28 years, with an age range of 29–68 years. Diabetes mellitus was present in 25 patients (58.1%), while hypertension was reported in 19 patients (44.2%). Fever was the most common clinical feature, occurring in 40 patients (93.0%), followed by vomiting in 16 patients (37.2%). Previous or prophylactic antibiotic use was reported in 28 patients (65.1%). The mean catheterization duration was 11.95 ± 3.63 days, while the mean ICU stay was 1.05 ± 1.45 days. The mean dialysis duration was 22.88 ± 10.05.

Table 2. Laboratory Findings of the Studied Patients

Laboratory parameter	Value
CRP mg/L Mean ± SD	38.35 ± 35.2
Serum creatinine,(mg/dL) Mean ± SD	7.17 ± 2.35
TLC(×10³/µL) Mean ± SD	16.47 ± 3.18
RBS(mg/dL) Mean ± SD	190.30 ± 78.31

Category variables are expressed as frequency (n) and percentage (%).

Continuous variables are expressed as Mean ± Standard Deviation (SD).

Laboratory investigations showed among the studied patients. The median CRP level was 48 mg/L (IQR: 24–72). The mean serum creatinine level was 7.17 ± 2.35 mg/dL, while the mean TLC was 16.47 ± 3.18 × 10³/µL. The mean RBS level was 190.30 ± 78.31 mg/dL.

Figure 1: Laboratory Findings of the Studied Patients

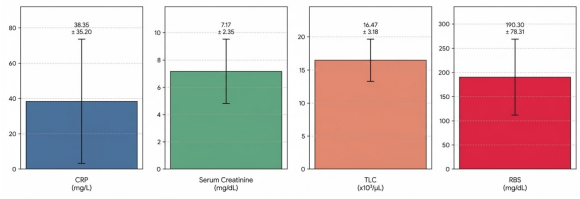


Table (3): Antimicrobial Susceptibility Profile of *Klebsiella pneumoniae* Isolates (n=43)

Antimicrobial	Susceptible (S)n(%)	Intermediate (I)n(%)	Non-susceptible n(%)
Ampicillin–sulbactam	14 (32.6%)	-	29 (67.4%)
Piperacillin	1 (2.3%)	2 (4.6%)	40 (93.0%)
Piperacillin–tazobactam	22 (51.2%)	-	21 (48.8%)
Cefazolin	4 (9.3%)	-	39 (90.7%)
Cefoxitin	21 (48.8%)	-	22 (51.2%)
Ceftazidime	8 (18.6%)	1 (2.3%)	34 (79.1%)
Ceftriaxone	8 (18.6%)	-	35 (81.4%)
Cefepime	7 (16.3%)	-	36 (83.7%)
Meropenem	15 (34.9%)	-	28 (65.1%)
Amikacin	22 (51.2%)	2 (4.7%)	19 (44.2%)
Gentamicin	16 (37.2%)	3 (7.0%)	24 (55.8%)
Tobramycin	7 (16.3%)	10 (23.3%)	26 (60.5%)
Ciprofloxacin	8 (18.6%)	4 (9.3%)	31 (72.1%)

Antimicrobial	Susceptible (S)n(%)	Intermediate (I)n(%)	Non-susceptible n(%)
Levofloxacin	5 (11.6%)	2 (4.7%)	38 (83.7%)*

Data are expressed as frequency (n) and percentage (%).

The antimicrobial susceptibility profile showed extensive resistance across several antibiotic classes. Complete resistance was observed to ampicillin (100.0%). High resistance rates were also observed for cefazolin (90.7%), levofloxacin (88.4%), ceftriaxone (86.0%), ceftazidime (83.7%), cefepime (83.7%), and imipenem (83.7%). Resistance to meropenem was 69.8%. Among the tested aminoglycosides, resistance was 55.8% for gentamicin and 46.5% for amikacin.

Table 4. MDRO and Antimicrobial Resistance Phenotypes among *K. pneumoniae* Isolates

Resistance Phenotype	Positive MDRO n(%)	Negative MDROn(%)	P-value
ESBL	34 (79.1%)	9 (20.9%)	**<0.001***
CRE	30 (69.8%)	13 (30.2%)	**0.010***
AmpC	27 (62.8%)	16 37.2	0.093

Data are represented as frequency (n) and percentage (%).

P-value was calculated using Chi-square test

Statistically significant (P < 0.05).

A high prevalence of antimicrobial resistance phenotypes was observed among the 43 *Klebsiella pneumoniae* isolates. ESBL production was detected in 34 isolates (79.1%), while CRE and AmpC phenotypes were identified in 30 (69.8%) and 27 (62.8%) isolates, respectively. These findings indicate a high burden of multidrug resistance and β-lactam resistance mechanisms among the studied *Klebsiella pneumoniae* isolates.

Figure 2: MDRO and Antimicrobial Resistance Phenotypes among *K. pneumoniae* Isolates

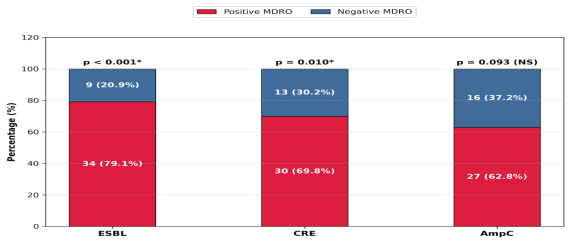


Table (5): Biofilm formation ability among *Klebsiella pneumoniae* isolates using Microtitre Plate assay (n=43)

Biofilm Assessment Category	Number (n)	Percentage (%)
Strong producer	17	39.5%
Moderate producer	17	39.5%
Weak producer	4	9.3%
Non-producer	5	11.6%

Data are represented as frequency (n) and percentage (%).

Non-biofilm producers: OD ≤ ODc

Weak biofilm producers: ODc < OD ≤ 2×ODc

Moderate biofilm producers: 2×ODc < OD ≤ 4×ODc

Strong biofilm producers: OD > 4×ODc

Biofilm formation was assessed using the microtiter plate (MTP) assay and Congo red agar (CRA) method. According to the MTP assay, biofilm production was detected in 38 of 43 isolates (88.4%). Strong and moderate biofilm production were each observed in 17 isolates (39.5%), while 4 isolates (9.3%) were weak biofilm producers and 5 isolates (11.6%) were classified as non-producers.

Figure (3): Biofilm formation ability among *Klebsiella pneumoniae* isolates using Microtitre Plate assay

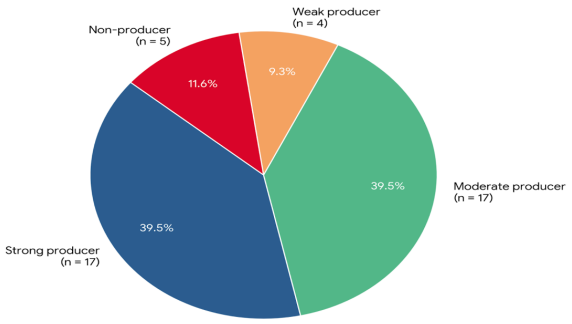


Table 6. Association between Biofilm Formation and Antimicrobial Resistance Phenotypes

Resistance phenotype	Biofilm positive,	Biofilm negative,	P-value
MDRO	38 (100.0%)	3 (60.0%)	0.011
ESBL	33 (86.8%)	1 (20.0%)	0.005
AmpC	26 (68.4%)	1 (20.0%)	0.056
CRE	28 (73.7%)	2 (40.0%)	0.153

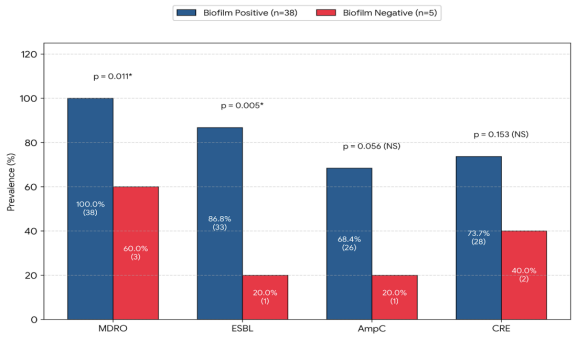
Data are presented as number (n) and percentage (%).

P-value was calculated using **Fisher's exact test**

Statistically significant difference (P < 0.05).

A significant association was observed between biofilm formation and both MDRO and ESBL phenotypes. All biofilm-positive isolates were classified as MDROs compared with 60.0% of biofilm-negative isolates (P = 0.011). Similarly, ESBL production was significantly more frequent among biofilm-positive isolates than among biofilm-negative isolates (86.8% vs. 20.0%, P = 0.005). AmpC production was more frequent among biofilm-positive isolates than among biofilm-negative isolates (68.4% vs. 20.0%), although the difference was not statistically significant (P = 0.056). CRE was also more frequent among biofilm-positive isolates than among biofilm-negative isolates (73.7% vs. 40.0%), but this difference was not statistically significant (P = 0.153).

Figure4:Association between Biofilm Formation and Antimicrobial Resistance Phenotypes



Discussion

Klebsiella pneumoniae bloodstream infections in hemodialysis patients represent a major clinical challenge because of antimicrobial resistance, including ESBL and AmpC production and carbapenem resistance (Cabrera et al., 2025; Shah et al., 2025; Shie et al., 2026). Central line-associated bloodstream infections (CLABSIs) are of particular concern in these patients because prolonged catheter use and patient-related risk factors may increase the risk of infection (Guo et al., 2024; Jia et al., 2026).

The study aimed to investigate antimicrobial resistance patterns and mechanisms, particularly ESBL, AmpC, and CRE, and to assess their association with biofilm formation in clinical *K. pneumoniae* isolates using microtiter plate (MTP) assays.

A high prevalence of antimicrobial resistance was observed among the *Klebsiella pneumoniae* isolates in the present study. ESBL production was detected in 79.1% of isolates, AmpC production in 62.8%, and carbapenem resistance (CRE) in 69.8%. These findings indicate a substantial burden of β-lactam resistance, which may considerably limit the effectiveness of commonly used cephalosporins and carbapenems and complicate the selection of appropriate antimicrobial therapy.

Ali et al. (2025), in a study of 200 *K. pneumoniae* isolates, also reported considerable ESBL and AmpC production, together with high resistance to third-generation cephalosporins. In contrast with our results, the prevalence of these resistance mechanisms was lower in their study. This difference may be explained by variations in patient populations, previous antimicrobial exposure, local antibiotic prescribing practices, and the epidemiological distribution of resistant strains.

Similarly, Şibar and Şencan (2026), who investigated catheter-related bloodstream infections in hemodialysis patients, reported carbapenem resistance in 18.2% of *Klebsiella* isolates. In contrast with our results, the carbapenem resistance rate in their study was considerably lower than the 69.8% observed in the present study. This marked difference may reflect variations in local antimicrobial selection pressure, infection-control practices, patient characteristics, and the circulation of carbapenem-resistant strains.

In the present study, amikacin and piperacillin–tazobactam showed the highest susceptibility rates among the tested antibiotics. Consistent with our findings**,** Li et al. (2024) identified amikacin and piperacillin–tazobactam as relatively active agents against *Klebsiella pneumoniae* isolates from hospitalized patients in China, although the susceptibility rates were higher (84.5% and 76.7%, respectively). This similarity in the relative activity of these antibiotics may support their potential role in the treatment of *K. pneumoniae* infections, while differences in susceptibility rates may reflect variations in patient populations, previous antimicrobial exposure, and local resistance patterns.

Regarding the resistance profile, the present study demonstrated high non-susceptibility to cefazolin (90.7%). These findings are consistent with those of Şibar and Şencan (2026), who reported considerable resistance to cephalosporins (63.6–72.7%) among *Klebsiella* isolates recovered from hemodialysis-related catheter bloodstream infections. these findings indicate a substantial burden of antimicrobial resistance among the *K. pneumoniae* isolates in the present study and support the need for local antimicrobial susceptibility surveillance to guide empirical treatment.

Biofilm formation was highly prevalent in the present study, with 88.4% of *K. pneumoniae* isolates classified as biofilm producers by the microtiter plate assay. Moreover, 79.0% demonstrated moderate or strong biofilm production. This finding is clinically relevant because biofilm formation facilitates bacterial persistence on indwelling devices and may reduce antimicrobial penetration and contribute to recurrent or difficult-to-treat catheter-associated infections. Shafaie et al. (2025) reported biofilm formation in 83.2% of 119 *K. pneumoniae* isolates, a prevalence comparable to that observed in our study.

The association between biofilm formation and antimicrobial resistance was particularly evident in the present study. All biofilm-positive isolates were MDR

klebsiella , and a significant association was observed between biofilm formation and both MDRO status and ESBL production. This finding agrees with Kafa et al. (2025), who reported substantially stronger biofilm formation among resistant *K. pneumoniae* isolates than among susceptible isolates. Similarly, Shafaie et al. (2025) found that biofilm-producing *K. pneumoniae* isolates had a higher frequency of multidrug resistance and demonstrated an association between biofilm formation and the ESBL.

The present findings are also supported by Nazari et al. (2024), who investigated 402 clinical *K. pneumoniae* isolates using the microtiter plate method and demonstrated a substantial burden of antimicrobial resistance together with biofilm formation. Their findings reinforce the importance of considering biofilm production when interpreting the clinical significance and treatment difficulty of resistant *K. pneumoniae* infections.

The clinical characteristics of these patients indicate a high-risk population, with frequent diabetes mellitus, prolonged catheterization, repeated hemodialysis exposure, and previous or prophylactic antibiotic use. These factors may contribute to both bloodstream infection and selection of resistant *K. pneumoniae* strains. This observation is consistent with Guo et al. (2024), whose meta-analysis of 49 studies identified diabetes, longer dialysis duration, prolonged catheter duration, hypertension, and elevated C-reactive protein among significant risk factors for catheter-associated bloodstream infection in hemodialysis patients. Similarly, Jia et al. (2026) identified diabetes mellitus and prolonged catheter indwelling time as independent risk factors for catheter-related bloodstream infection in patients undergoing hemodialysis through central venous catheters.

The elevated CRP and leukocyte count, supports the presence of clinically significant infection rather than simple catheter colonization. Guo et al. (2024) also reported a significant association between CRP and catheter-associated bloodstream infection in hemodialysis patients. In addition, Şibar and Şencan (2026) found that CRP and white blood cell counts were significantly elevated in patients with severe outcomes of hemodialysis-associated catheter bloodstream infection, emphasizing the clinical value of inflammatory markers in this population.

These findings emphasize the importance of local antimicrobial susceptibility surveillance, appropriate empirical therapy based on institutional resistance patterns, antimicrobial stewardship, and strict catheter-care practices. In patients requiring central venous access for hemodialysis, prevention of catheter infection and early recognition of resistant *K. pneumoniae* are essential to improve treatment outcomes and limit further dissemination of antimicrobial resistance.

5. CONCLUSION

The present study identified a strong relationship between biofilm formation and multi drug resistance in *K. pneumoniae*, particularly ESBL. The coexistence of biofilm formation and multidrug resistance may contribute to bacterial persistence and reduced treatment effectiveness. These findings support the need for therapeutic approaches capable of addressing both resistance mechanisms and the biofilm barrier and strict infection controle measure .

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