IKR Publishers

IKR Journal of Clinical Medicine and Medical Research (IKRJCMMR)
Journal homepage: https://ikrpublishers.com/ikrjcmmr/
Volume-1, Issue-2 (July-August) 2025
OPEN ACCESS
ISSN: 3107-6521 (Online)

Synergistic Roles of Quorum Sensing, Biofilm Formation, and Integron-Mediated Resistance in the Infection of Children's Klebsiella Pneumoniae

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DOI:10.5281/zenodo.17597494

ARTICLE INFO

Article history:
Received : 20-10-2025
Accepted : 01-11-2025
Available online : 13-11-2025

Citation: Al-Ezee, A. S. (2025). Synergistic Roles of Quorum Sensing, Biofilm Formation, and Integron-Mediated Resistance in the Infection of Children's Klebsiella Pneumoniae. *IKR Journal of Clinical Medicine and Medical Research (IKRJCMMR)*, 1(2), 32-37.

ABSTRACT **Original research paper**

The present study investigated the virulence determinants and antibiotic resistance patterns of *Klebsiella pneumoniae* isolates recovered from pediatric stool samples in Iraq. Out of 94 samples, 17 isolates (13.6%) were confirmed using biochemical identification and 16S rRNA gene sequencing. These isolates demonstrated high virulence potential, where 92% produced quorum-sensing N-acyl homoserine lactone (AHL) signals and 98% carried the sdiA gene. Biofilm formation was detected in all isolates, with 76% exhibiting strong biofilm production. Hemolysin activity was observed in 64% of isolates, indicating enhanced persistence and potential tissue damage.

Antimicrobial susceptibility testing revealed high levels of resistance to β-lactam antibiotics, particularly ampicillin (96%), amoxicillin-clavulanate (94%), cefotaxime (92%), and ceftazidime (88%). In contrast, imipenem (14%) and tigecycline (0% resistance) retained notable effectiveness.

Extended-spectrum β-lactamase (ESBL) production was detected in 18% of isolates, while metallo-β-lactamase (MBL) production was found in 46%. Integron analysis showed the presence of class 1 and class 2 integrons in 66% and 18% of isolates, respectively. Additionally, sul1 and qacEΔ1 genes were identified in 67.6% and 85.3% of integron-positive isolates, respectively. Overall, 96% of isolates were categorized as multidrug-resistant (MDR) and 20% as extensively drug-resistant (XDR).

These findings highlight the coexistence of virulence and resistance determinants in pediatric *Klebsiella pneumoniae*, emphasizing the urgent need to strengthen infection control practices, improve molecular surveillance systems, and promote responsible antimicrobial use to limit the spread of highly resistant and virulent strains in clinical settings.

Keywords: *Klebsiella pneumoniae*, Pediatric Infections, Biofilm Formation, Quorum Sensing, Integrons, (MDR), (XDR).

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1. Introduction

Klebsiella pneumoniae is a clinically important Gram-negative pathogen within the family *Enterobacteriaceae*. It is frequently associated with respiratory tract infections, urinary tract infections, bacteremia, and gastrointestinal infections (1). Its medical importance is further intensified in hospital environments, where it represents a major cause of healthcare-associated infections, particularly among neonatal, pediatric, and intensive care unit patients (2).

In recent years, antimicrobial resistance in *K. pneumoniae* has risen sharply, leading to the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and, in some cases, pandrug-resistant (PDR) strains (3). These resistance phenotypes are often mediated by mobile genetic elements, including plasmids and integrons, which facilitate horizontal gene transfer and rapid dissemination of resistance traits (4). As a result, the World Health Organization has classified *K. pneumoniae* as a critical-priority pathogen due to its frequent

resistance to β-lactams, carbapenems, and fluoroquinolones (2).

Alongside resistance, *K. pneumoniae* expresses several virulence determinants that contribute to infection persistence and severity. These include biofilm formation, which enhances tolerance to antimicrobials and disinfectants (5), hemolysin-mediated erythrocyte lysis that promotes tissue damage (6), and quorum-sensing systems that coordinate the expression of multiple virulence genes (7). Recent surveillance studies in Iraq have reported high levels of ESBL and carbapenem resistance among *K. pneumoniae* isolates (8), with similar resistance trends reported across neighboring countries, indicating a broader regional public health concern (9,10).

Therefore, the present study aims to isolate and identify *K. pneumoniae* from pediatric stool samples, determine key virulence traits (biofilm formation, hemolysin production, quorum-sensing signaling), assess antimicrobial resistance patterns, and detect integrons and their associated resistance genes.

2. Materials and Methods

The present study was conducted at Al-Batool Maternity and Children's Hospital, in collaboration with affiliated private diagnostic laboratories in Diyala, Iraq, during the period from November 2024 to April 2025. A total of 94 stool samples were collected from pediatric patients aged between one and seven years. Sterile, leak-proof containers were used for sample collection, and samples were transported under refrigerated conditions at 4°C and processed within 2–4 hours to ensure bacterial viability (1).

For microbiological isolation, samples were cultured on blood agar, MacConkey agar, and eosin methylene blue (EMB) agar plates following standard laboratory procedures (2). Presumptive identification of *K. pneumoniae* was based on the observation of mucoid, lactose-fermenting colonies, followed by confirmation using biochemical tests such as urease and citrate utilization (3).

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (5) guidelines. The antibiotic panel included β-lactams, aminoglycosides, macrolides, fluoroquinolones, tetracyclines, carbapenems, and sulfonamides. The minimum inhibitory concentration (MIC) of polymyxin B was determined via broth microdilution (4).

Biofilm formation capability was assessed using the crystal violet microtiter plate assay, and optical density was measured at 570 nm (5). Hemolysin activity was evaluated using 5% sheep blood agar, where complete and partial hemolysis were indicated by clear and greenish zones, respectively (6).

Phenotypic detection of extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) production was carried out using the double-disk synergy test (DDST) and the imipenem–EDTA combined disk method, respectively (7,8). Quorum-sensing activity was examined by ELISA-based detection of N-acyl homoserine lactone (AHL) signaling molecules, and the presence of the sdiA regulatory gene was confirmed by PCR (9,10).

Genomic DNA extraction was performed using a commercial spin-column purification kit containing Proteinase K, following the manufacturer's instructions. DNA purity was assessed using a NanoDrop spectrophotometer, with acceptable A260/A280 values ranging from 1.8 to 2.0 (11). Primers targeting intI1, intI2, intI3, 5′CS–3′CS, sul1, and qacEΔ1 genes were designed using NCBI reference sequences and validated via BLAST alignment (12,13). PCR amplicons were resolved on 1.5% agarose gels, visualized under UV illumination, and selected PCR products were sequenced to confirm gene identity (14).

Table 1. Represents the Primers Used in the Study

Primers ID	Gene	Nucleotide sequence (5′–3′)	size (bp)	Annealing temperature	References
	16 sRNA	F : AGAGTTTGATCCTGGCTCAG	1500	58 °C	(12)
		R: GGTTACCTTGTTACGACTT			
Q. S.	*sdiA*	F :TGCAACGGGAAAAGGACAA	66	68 °C	(10)
		R:GCGGTGTCACTCAGTATTTAATGC			
Integron Genes	*intl*	F: CCTCCCGCACGATGATC	280	58 °C	(12)
		R: TCCACGCATCGTCAGGC			
	intll	F: TTATTGCTGGGATTAGGC	300	51 °C	
		R: ACGGCTACCCTCTGTTATC			
	Intl 3	F: AGTGGGTGGCGAATGAGTG	300	51 °C	
		R: TGTTCTTGTATCGGCAGGTG			
Gen resestance	*sul*1	F: CGGCGTGGGCTACCTGAACG	433	67 °C	(27)
		R: GCCGATCGCGTGAAGTTCCG			
	*qac*EΔ1	F: ATCGCAATAGTTGGCGAAGT	250	58 °C	
		R: GAAGCTTTTGCCCATGAAGC			

3. Results

3.1 Prevalence of *Klebsiella pneumoniae*

Out of the 94 stool samples collected from pediatric patients, 17 isolates (18.1%) were confirmed as *Klebsiella pneumoniae* using conventional biochemical profiling and 16S rRNA gene sequencing, ensuring high diagnostic reliability. This prevalence indicates a notable carriage rate of *K. pneumoniae* in children and underscores its clinical relevance as an enteric pathogen. Comparable prevalence levels have been documented in previous investigations conducted in Iraq and other countries, including India and China, reflecting the widespread epidemiological significance of this pathogen (1–4).

3.2 Quorum Sensing Activity

Quorum-sensing activity was detected in 94% of the isolates based on ELISA quantification of N-acyl homoserine lactone (AHL) molecules, while the sdiA regulatory gene was identified in all isolates (100%). These findings suggest robust quorum-sensing-mediated regulatory mechanisms that facilitate virulence expression and environmental adaptability, particularly in hospital-associated strains. Previous studies similarly link quorum sensing with increased pathogenicity and antimicrobial resistance (5,6).

3.3 Biofilm Formation

All isolates (100%) were capable of forming biofilms. Among them, 76% exhibited strong biofilm production and 24% exhibited moderate levels. Biofilm formation represents a key virulence strategy contributing to antimicrobial tolerance and persistent colonization. These results correspond with national and international reports demonstrating an association between strong biofilm phenotypes and MDR/XDR resistance profiles in *K. pneumoniae* (7).Table 2

Table 2. Biofilm Formation Strength of Pediatric *Klebsiella pneumoniae*

Biofilm Strength	Number of isolates (n=17)	%
Strong	13	76%
Moderate	4	24%
Weak/None	0	0%

3.4 Hemolysin Production

Hemolysin activity was detected in 11 isolates (64.7%). The presence of hemolysin indicates an enhanced capacity for erythrocyte lysis and tissue damage, contributing to disease severity, particularly in invasive infections (8).Table 3

Table 3. Hemolysin Production in Pediatric *Klebsiella pneumoniae* Isolates

Hemolysin Activity	Number of isolates (n=17)	%
Positive	11	65%
Negative	6	35%

3.5 ESBL and MBL Production

Phenotypic testing revealed that 3 isolates (18%) produced extended-spectrum β-lactamases (ESBLs), while 8 isolates (47%) produced metallo-β-lactamases (MBLs). These enzyme-mediated mechanisms significantly undermine the therapeutic efficacy of β-lactam antibiotics, including cephalosporins and carbapenems. Similar resistance patterns have been previously documented in Iraq, Libya, and Iran, and are recognized globally as major clinical challenges (3,9).

3.6 Antibiotic Resistance Patterns

High resistance rates were observed to β-lactam antibiotics, including ampicillin (94%), amoxicillin-clavulanate (94%), cefotaxime (88%), and ceftazidime (88%). Resistance to imipenem was lower (12%), while tigecycline retained complete activity (0% resistance). These trends highlight the limited therapeutic options and designate carbapenems and tigecycline as last-resort agents. Comparable resistance patterns have been reported globally, although regional differences persist (10–13).Table 1.

3.7 Minimum Inhibitory Concentration (MIC) of Polymyxin B

The MIC of polymyxin B ranged from 2–4 µg/mL, with 8 isolates (47%) exhibiting resistance. Given that polymyxins represent salvage therapy for severe MDR infections, the emergence of resistance is clinically alarming and may compromise future treatment outcomes (14).Table 4.

Table 4. Antibiotic Resistance Patterns of Pediatric Klebsiella pneumoniae

Antibiotic	Resistant isolates (n=17)	% Resistance
Ampicillin	16	94%
Amoxicillin-Clavulanate	16	94%
Cefotaxime	15	88%
Ceftazidime	15	88%
Imipenem	2	12%
Tigecycline	0	0%
Polymyxin B (MIC 2-4 µg/mL)	8	47%

3.8 MDR and XDR Classification

Based on antimicrobial susceptibility profiles, 16 isolates (94%) were classified as multidrug-resistant (MDR) and 4 isolates (23.5%) as extensively drug-resistant (XDR). These rates exceed global estimates, which typically range between 60–80% for MDR *K. pneumoniae*, highlighting a critical antimicrobial resistance burden in the region (15).Table 5. Figure 1.

Table 5. Distribution of Integrons Among Pediatric *Klebsiella pneumoniae*

Integron Class	Number of isolates (n=17)	%
Class 1	13	76.4%
Class 2	3	17.6%
Class 3	1	5.8%

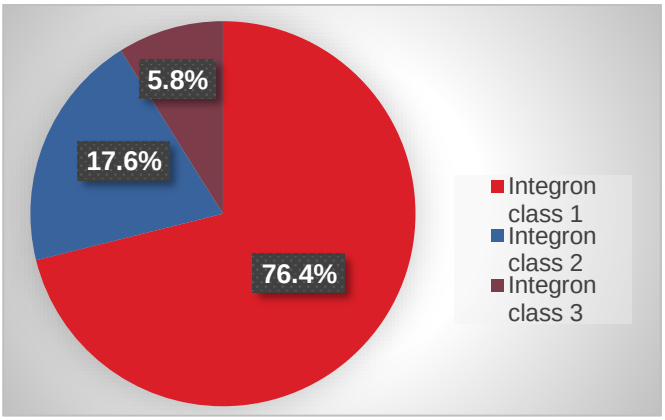

Figure 1. Shows the Distribution of Integron Types Among the Isolates

3.9 Integrons and Associated Genes

Integron analysis revealed that 13 isolates (76.4%) carried class 1 integrons, 3 isolates (17.6%) carried class 2 integrons, and 1 isolate (5.8%) carried class 3 integrons. Among class 1 integron carriers, sul1 was detected in 11 isolates (64.7%), and qacEΔ1 was detected in 14 isolates (82.3%). These findings substantiate the role of integrons in mediating the dissemination of antimicrobial resistance determinants, consistent with previous regional studies (3).Table 6

Table 6. Presence of Resistance Genes Among Class 1 Integron-Carrying

Resistance Gene	Number of isolates (n=13)	%
sul1	11	84.6%
qacEΔ1	14	82.3%

4. Discussion

The findings of the present study demonstrate that pediatric *Klebsiella pneumoniae* isolates in Iraq possess a considerable virulence potential characterized by multiple, interrelated pathogenicity mechanisms. A high proportion of isolates exhibited active quorum-sensing (QS) systems, as evidenced by detectable AHL signaling molecules and the universal presence of the **sdiA** gene, which is known to play a pivotal regulatory role in the coordination of virulence-associated gene expression and adaptive responses to environmental stressors, particularly those encountered in hospital settings. This regulatory capacity likely contributes to the robust biofilm formation observed in all isolates, with a large subset demonstrating strong biofilm production. Biofilm-mediated community organization provides structural stability and confers protection against host immune responses, antimicrobial agents, and disinfectants, thereby facilitating persistent colonization and survival within clinical environments.

Additionally, hemolysin activity was detected in a substantial proportion of isolates, indicating an enhanced capacity to damage host tissues and exacerbate disease severity. When considered alongside the observed production of extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs), which significantly restrict therapeutic options, the clinical management of infections caused by these isolates becomes increasingly challenging. Antimicrobial susceptibility testing revealed high resistance rates to commonly used treatment regimens, with worrisome resistance even to last-line agents such as polymyxin B. The concurrence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes with prominent virulence determinants suggests a synergistic interaction that enhances both pathogenicity and persistence.

Molecular characterization further indicated a high prevalence of integrons, particularly class 1 integrons harboring sul1 and qacEΔ1, which facilitate horizontal gene transfer and accelerate the dissemination of resistance determinants across bacterial populations. The integration of these mobile genetic elements with QS-regulated behavior, biofilm-associated tolerance, and β-lactamase-mediated enzymatic inactivation reflects the presence of a complex, multifactorial resistance–virulence network that promotes survival, adaptability, and pathogenic success.

In summary, pediatric *K. pneumoniae* isolates in Iraq exhibit a coordinated expression of virulence and resistance mechanisms, including quorum sensing, extensive biofilm formation, hemolysin production, β-lactamase activity, MDR/XDR phenotypes, and integron-mediated genetic exchange. These findings underscore an urgent need for reinforced infection control strategies, optimized antimicrobial stewardship programs, and continued molecular surveillance to mitigate the emergence and spread of highly virulent and drug-resistant *K. pneumoniae* strains within pediatric healthcare settings.

5. Conclusion and Recommendations

This study demonstrates a notably high prevalence of *Klebsiella pneumoniae* among pediatric stool samples in Diyala, Iraq, accompanied by a striking profile of antimicrobial resistance. The isolates exhibited elevated rates of multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes, along with pronounced virulence attributes including strong biofilm formation, hemolysin production, and active quorum-sensing mechanisms. Furthermore, the detection of integrons—particularly class 1—and associated resistance determinants such as **sul1** and **qacEΔ1** underscores the exceptional capacity of these isolates to acquire, disseminate, and stabilize antimicrobial resistance through horizontal gene transfer.

The widespread resistance observed to β-lactams, cephalosporins, and even carbapenems, along with the emergence of resistance to polymyxin B—considered a last-resort therapeutic agent—poses a critical clinical challenge. These findings reflect not only the global escalation of antimicrobial resistance but also highlight distinct regional risks that warrant immediate attention.

Accordingly, stringent infection prevention strategies, reinforcement of antimicrobial stewardship programs, and

continuous molecular surveillance are imperative to curtail the transmission and evolution of resistant *K. pneumoniae* strains. Future research should prioritize genomic and functional analyses to elucidate the regulatory interplay between quorum-sensing pathways, biofilm-associated genes, and resistance determinants.

In conclusion, *K. pneumoniae* represents a high-priority clinical pathogen due to the convergence of potent virulence mechanisms and extensive resistance profiles. Addressing its clinical and epidemiological consequences necessitates sustained monitoring, rational antimicrobial utilization, and improved diagnostic and infection-control frameworks.

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