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RESEARCH ARTICLE OPEN ACCESS

Molecular detection of virulence and carbapenem resistance genes in clinical isolates of Pseudomonas Aeruginosa from Iraqi hospitals

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Abstract
Objective: This study aimed to assess the molecular prevalence of six key resistance and virulence genes, toxA, oprL, KPC, vim2, hlyA, and vanB2, in Pseudomonas aeruginosa isolates obtained from clinical sources in selected Iraqi hospitals.
Methods: A total of 200 clinical specimens were collected from three hospitals in Iraq. Of these, 25 isolates were confirmed as P. aeruginosa based on culture and biochemical identification. All 25 isolates were subjected to polymerase chain reaction (PCR) to detect the six target genes. PCR amplification products were analysed by gel electrophoresis. Statistical associations between gene presence and sample sources were evaluated using chi-square tests.
Results: All 25 isolates tested positive for toxA and oprL, indicating their ubiquity and potential utility as diagnostic markers. The KPC gene was detected in 84% (21/25) of the isolates, highlighting a high prevalence of carbapenem resistance. No amplification was observed for vim2, hlyA, or vanB2 in any of the isolates. Significant associations were found between gene presence and hospital or specimen source ($\chi^2 = 136.52$, $p < 0.00001$).
Conclusion: The detection of KPC alongside the universal presence of toxA and oprL genes underscores the clinical importance of these markers in Iraqi hospital settings. The absence of vim2, hlyA, and vanB2 may reflect regional genomic patterns. These findings emphasise the need for molecular diagnostics, enhanced infection control, and ongoing antimicrobial resistance surveillance to inform treatment protocols and policy.

Keywords: Pseudomonas aeruginosa, Drug resistance, Virulence factors, Polymerase Chain Reaction, Iraq

Plain English Summary

This study examined a type of hospital-acquired bacterium known as Pseudomonas aeruginosa, which can cause severe infections, particularly in individuals with weakened immune systems. Researchers collected 200 clinical samples from three hospitals in Iraq. Of these, 25 samples were confirmed to contain P. aeruginosa. The goal was to determine whether these bacteria carried specific genes that make them more harmful or resistant to treatment. All 25 isolates had two important genes, toxA and oprL, which help the bacteria damage human cells and survive in tough environments. Most concerning was the finding that 84% of the bacteria had a gene called KPC, which makes them resistant to powerful antibiotics known as carbapenems. These are often considered “last resort” antibiotics used when other drugs fail.

Three other genes (vim2, hlyA, and vanB2), which are sometimes linked to antibiotic resistance in other settings, were not found in any of the samples. This research shows that P. aeruginosa in Iraqi hospitals may be especially hard to treat due to the presence of these resistance genes. It highlights the need

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for better hospital infection control, cautious use of antibiotics, and regular testing to keep track of how these bacteria are changing over time.

Introduction

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, obligate aerobic bacterium that does not ferment lactose and is oxidase- and catalase-positive. It is widely distributed in natural and man-made environments, including soil, water, and moist surfaces such as hospital sinks, respiratory equipment, and catheters. Clinically, P. aeruginosa poses a major challenge due to its intrinsic resistance to many antibiotics and its ability to acquire additional resistance and virulence determinants, making it a frequent cause of difficult-to-treat healthcare-associated infections, particularly in immunocompromised patients (1).

In the Middle East, including Iraq, the burden of multidrug-resistant (MDR) and extensively drug-resistant (XDR) P. aeruginosa infections is rising, with reports highlighting high resistance rates to cephalosporins, fluoroquinolones, and carbapenems (2, 3, 4). Iraqi hospitals have reported MDR rates exceeding 70% and increasing prevalence of resistance genes, reflecting a serious threat to infection control and treatment outcomes (3, 5). These trends underscore the urgent need for localised molecular surveillance to inform diagnostics, treatment protocols, and antimicrobial stewardship.

The virulence of P. aeruginosa is largely attributed to its capacity to produce multiple pathogenic factors, including exotoxins, proteases, haemolysins, and biofilm components. Among the most well-characterised virulence determinants is the toxA gene, which encodes exotoxin A, a potent inhibitor of protein synthesis in host cells. Studies across the region have documented toxA detection in over 90% of clinical isolates, including 100% of XDR strains in Egypt and 94.7% of clinical isolates in Baghdad (5, 6, 7).

Another key genetic marker is the oprL gene, which encodes an outer membrane lipoprotein involved in maintaining cell envelope integrity. It is highly conserved among P. aeruginosa strains and widely used in PCR-based species confirmation. Multiple studies have reported oprL detection in 80–100% of clinical isolates (8, 9).

Carbapenem resistance, a growing concern in clinical settings, is often mediated by carbapenemase genes. The KPC gene (Klebsiella pneumoniae carbapenemase), though originally associated with Enterobacteriaceae, has increasingly been detected in P. aeruginosa high-risk clones such as ST463. Iraqi studies report variable KPC prevalence, from 22.9% to over 40%, with higher rates observed in southern and central regions (2, 3). This study aims to further characterise the frequency of KPC in local isolates, given its critical role in carbapenem resistance.

In addition to these well-established genes, we included three other genetic markers in our analysis: vim2, hlyA, and vanB2. While their association with P. aeruginosa is less well-defined, there is emerging evidence suggesting potential relevance in certain clinical contexts. The vim2 gene encodes a metallo-β-lactamase and has been reported in up to 60% of carbapenem-resistant isolates in some countries, though with limited data from Iraq (10, 11). The hlyA gene, typically linked to haemolysin production in other Gram-negative bacteria, has been occasionally reported in P. aeruginosa isolates with haemolytic phenotypes, suggesting possible horizontal gene acquisition or misattributed virulence roles (12, 13). Similarly, vanB2, known for mediating vancomycin resistance in Enterococci, has been controversially identified in P. aeruginosa isolates, possibly indicating alternative resistance mechanisms or contamination (14, 15).

Given these regional trends and uncertainties, the present study aims to assess the molecular prevalence of six key genes, toxA, oprL, KPC, vim2, hlyA, and vanB2, among P. aeruginosa isolates obtained from clinical specimens in major Iraqi hospitals. By correlating these findings with regional and global data, we hope to advance understanding of the evolving virulence and resistance landscape of P. aeruginosa in Iraq.

Materials and Methods

Study Design and Sample Collection

This cross-sectional study was conducted between January and August 2024. A total of 200 clinical specimens were collected from patients presenting with suspected infections at three major hospitals in Iraq: Al-Sadr Teaching Hospital (Najaf), Burns Hospital (Diwaniyah), and Hilla Teaching Hospital. Specimens were obtained aseptically from wounds, burns, and ear discharges and immediately transported to the microbiology laboratory for processing within two hours of collection.

The inclusion criteria included clinical suspicion of infection and availability of adequate specimen volume. Exclusion criteria were samples with contamination, insufficient volume, or delayed transport exceeding two hours. Of the 200 collected specimens, only 25 were confirmed as

Pseudomonas aeruginosa through culture and biochemical identification.

Bacterial Isolation and Identification
Specimens were cultured on Cetrimide and Nutrient agar and incubated aerobically at 37 °C for 24 to 48 hours. Colonies exhibiting characteristic green pigmentation and grape-like odour were selected and subjected to standard biochemical tests, including Gram staining, oxidase, catalase, and citrate utilisation. Confirmed P. aeruginosa isolates were preserved in 20% glycerol at −20 °C until molecular testing.

Isolate Selection and Antibiotic Susceptibility Testing
From the 25 confirmed P. aeruginosa isolates, all were included for molecular analysis. These isolates were randomly selected using stratified sampling based on hospital source and specimen type to ensure representation across clinical settings. Phenotypic antibiotic susceptibility testing was not performed in this study; the focus was limited to genotypic characterisation.

DNA Extraction
Genomic DNA was extracted from overnight cultures using a commercial DNA extraction kit (manufacturer details), following the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer.

PCR Amplification of Target Genes
Polymerase Chain Reaction (PCR) was used to amplify six target genes: toxA, oprL, KPC, vim2, hlyA, and vanB2. Each 25 µL reaction mixture contained 1 µL genomic DNA, 1 µL of each forward and reverse primer, 12.5 µL PCR Master Mix (Taq DNA polymerase, dNTPs, MgCl2, and buffer), and nuclease-free water. The thermal cycling conditions included initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at gene-specific temperatures, and extension at 72 °C for 1 minute, ending with a final extension at 72 °C for 5 minutes (Table 1).

Table 1: Primer Sequences and PCR Conditions

Gene	Forward Primer (5′→2′)	Reverse Primer (5′→2′)	Product Size (bp)	Annealing Temp (°C)	Reference
toxA	GGTAACCAGCTCAGCCACAT	TGATGTCCAGGTCATGCTTC	352	57	(16)
oprL	ATGGAAATGCTGAAATTCGGC	CTTCTTCAGCTCGACGCGACG	504	57–58	(17)
KPC	ATGTCACTGTATCGCCGTCT	TTTTCAGAGCCTTACTGCCC	270	62	(18)
vim2	GGAGTCAGAGGATCGAGAAG	AGGCAGCAGTATCGAGTGTT	390	58	(10)
hlyA	ACAGGCAAGCCTGTAAGCG	AGCGTGTGTTCCTGTTCCAG	1170	60	(13)
vanB2	ATGGGAAGCCGATAGTCAGAG	TTACGCTGGCCTTCCGAAAAC	635	59	(15)

Positive and Negative Controls
Each PCR run included positive control DNA (where available) and a no-template negative control to ensure amplification specificity and exclude contamination.

Gel Electrophoresis
PCR products were separated by electrophoresis on 1.5% agarose gels stained with ethidium bromide. Gels were run at 100 V for 45 minutes and visualised under UV transillumination. A 100 bp molecular weight ladder was used as a size reference.

Statistical Analysis
Data analysis was performed using SPSS version 25. Frequencies and percentages were calculated for gene distributions. Chi-square (χ^2) tests were conducted to evaluate associations between gene presence and sample sources (e.g., wound, burn, ear) and hospital origin. A p-value of <0.05 was considered statistically significant.

Results
Gene Detection Profile in P. aeruginosa Isolates
All 25 confirmed P. aeruginosa isolates were screened for six target genes. The presence of toxA, oprL, and KPC genes was high, with toxA and oprL detected in 100% of the isolates and KPC found in 84% (21/25). In contrast, vim2, hlyA, and vanB2 genes were not detected in any of the isolates (Table 2).

Table 2: Summary of Gene Detection in P. aeruginosa Isolates (n=25)

Gene	Product Size (bp)	Positive Isolates (n)	Detection Rate (%)
toxA	352	25	100
oprL	504	25	100

KPC	270	21	84
vim2	390	0	0
hlyA	1170	0	0
vanB2	635	0	0

The gel electrophoresis (Figure 1) visually confirms the amplification of toxA, oprL, and KPC in positive isolates, with clear bands observed at their respective product sizes.

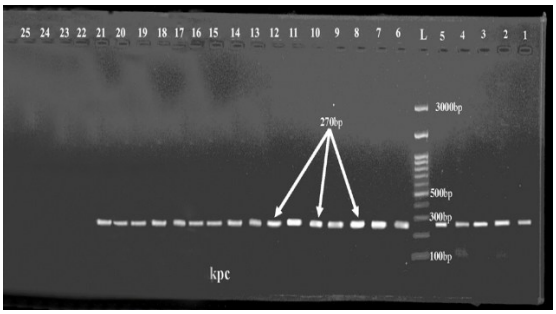


Figure 1: Agarose gel image showing PCR amplification of toxA, oprL, and KPC genes in representative isolates

Bands were visualised at expected product sizes (352 bp, 504 bp, and 270 bp, respectively). L: 100 bp DNA ladder

The histogram (Figure 2) and heat map (Figure 3) illustrate the relative frequency of each gene among the 25 isolates, highlighting complete detection of toxA and oprL, and high prevalence of KPC.

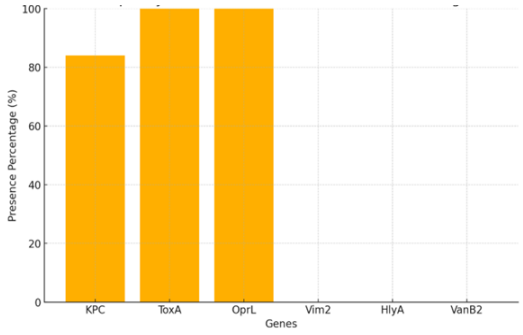


Figure 2: Histogram showing percentage distribution of gene positivity in the P. aeruginosa isolates

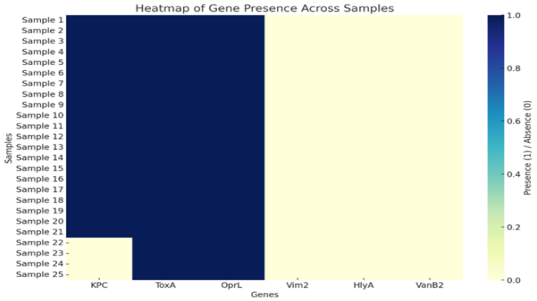


Figure 3: Heatmap of gene presence (1) and absence (0) across the 25 isolates

Chi-square tests revealed statistically significant differences in the presence of specific genes across sample types and hospital locations ($\chi^2 = 136.52$, $p < 0.00001$), indicating potential variation in virulence and resistance gene profiles based on clinical source and geographic location.

Discussion

The findings from this study reaffirm the clinical importance of toxA, oprL, and KPC genes in P. aeruginosa isolates from Iraqi hospitals. The universal detection of toxA and oprL aligns with previous studies in Iraq, Egypt, and Iran, confirming their high conservation and diagnostic

utility (5, 6, 7, 8, 9). Their consistent presence across diverse clinical specimens highlights their potential as reliable molecular markers for identifying virulent strains.

The detection of the KPC gene in 84% of isolates is particularly concerning, as this gene confers resistance to carbapenems such as imipenem, meropenem, and ertapenem, which are critical antibiotics in managing P. aeruginosa infections. This frequency exceeds reported rates from earlier studies in Basra and Baghdad, which documented KPC prevalence around 22.9%–40% (2, 3). Several factors may account for this discrepancy, including differences in patient population, hospital antibiotic use policies, or the spread of specific high-risk clones like ST463. The high prevalence of KPC-producing isolates underscores the urgency of implementing carbapenem stewardship programs and surveillance of plasmid-mediated resistance.

Globally, KPC-producing P. aeruginosa remains unevenly distributed, with much lower prevalence reported in regions like Japan (4.2%) (19), suggesting a complex interplay of geographic, genetic, and ecological factors (20). The emergence of multidrug-resistant clones like ST235, ST175, and ST463 in various countries highlights the role of clonal expansion and horizontal gene transfer in shaping local epidemiology (21, 22, 23, 24).

The complete absence of vim2, hlyA, and vanB2 in this study may reflect regional patterns or the limited role of these genes in local isolates. Although vim2 is commonly identified in Mediterranean and Asian settings (10, 11), its lack of detection here aligns with some Iraqi data suggesting dominance of non-metallo-β-lactamase resistance mechanisms. Similarly, hlyA and vanB2 have uncertain roles in P. aeruginosa, and their absence supports the view that phenotypic haemolysin activity and vancomycin resistance in this species likely arise from alternative pathways (12, 13, 14, 15, 25).

Therapeutic and Infection Control Implications

The high prevalence of virulent and resistant genes in P. aeruginosa isolates in this study necessitates urgent clinical and policy interventions. First, molecular diagnostics targeting toxA, oprL, and KPC should be incorporated into routine microbiology workflows to facilitate early identification of high-risk isolates. Second, hospitals in Iraq should strengthen antimicrobial stewardship programs with a particular focus on carbapenems and other critical drugs.

Third, infection control protocols should emphasise strict hygiene measures, environmental decontamination, and contact precautions in intensive care and burn units, where P. aeruginosa frequently colonises patients and equipment. Surveillance of resistance gene dissemination through mobile genetic elements is also warranted, particularly considering rising KPC prevalence.

Lastly, therapeutic regimens may need revision. Given the resistance conferred by KPC, newer antibiotics such as ceftazidime-avibactam or ceftolozane-tazobactam may offer more reliable options and should be evaluated in local clinical trials.

These findings highlight the urgent need to revise infection control policies and treatment guidelines in Iraq to address the high burden of carbapenem-resistant P. aeruginosa, with a focus on routine molecular surveillance, restricted carbapenem use, and implementation of targeted antimicrobial stewardship programs."

Study limitations

This study has several limitations. The sample size was relatively small (n=25), limiting generalizability. The isolates were drawn from only three hospitals in Iraq, which may introduce sampling bias and not reflect national trends. Furthermore, the study focused solely on genotypic detection of resistance and virulence genes; no phenotypic antibiotic susceptibility testing was performed, preventing correlation of gene presence with actual resistance patterns. Future studies should integrate whole-genome sequencing, antimicrobial susceptibility profiles, and broader geographic sampling to validate and extend these findings.

Conclusion

This study provides important molecular insights into the virulence and resistance gene profiles of P. aeruginosa clinical isolates from major Iraqi hospitals. The universal presence of toxA and oprL and the high prevalence of the KPC gene reinforce the clinical threat posed by these isolates, particularly in the context of limited therapeutic options. The absence of vim2, hlyA, and vanB2 may indicate a localised resistance profile distinct from other regional and global settings. These findings emphasise the necessity for targeted infection control practices, the integration of molecular diagnostics into routine clinical workflows, and the consideration of newer antimicrobial agents in treatment strategies.

Considering the escalating antimicrobial resistance crisis, this study underscores the need for ongoing surveillance programs across Iraq. Sustained molecular monitoring and broader multicentre studies are essential to track emerging resistance trends, guide evidence-based policy, and ensure an effective public health response.

This study highlights the urgent need for ongoing molecular surveillance of antimicrobial resistance (AMR) in Iraq. As resistance genes continue to evolve and spread, continuous monitoring of virulence and resistance patterns is essential to inform clinical practice, update treatment guidelines, and implement targeted infection control strategies.

List of Abbreviations

AMR: Antimicrobial Resistance
DNA: Deoxyribonucleic Acid
KPC: Klebsiella pneumoniae Carbapenemase
MDR: Multidrug-Resistant
PCR: Polymerase Chain Reaction
ST: Sequence Type
toxA: Exotoxin A Gene
oprL: Outer Membrane Lipoprotein L Gene
vim2: Verona Integron-Encoded Metallo-β-Lactamase 2
hlyA: Haemolysin A Gene
vanB2: Vancomycin Resistance Gene B2
XDR: Extensively Drug-Resistant

Declarations

Ethics Approval and Consent to Participate

This study was conducted following the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Research Ethics Committee of the College of Education, University of Al-Qadisiyah, Iraq (Approval No.: EDU/BIO/38-2024). Before specimen collection, verbal and/or written informed consent was obtained from all participants or their legal guardians per institutional policies. Patient confidentiality and data anonymity were strictly upheld throughout the study.

Consent for Publication

All authors provided consent for the publication of this manuscript under the Creative Commons Attribution Non-Commercial 4.0 International License.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no competing interests.

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Authors' Contributions

SZO: Conceptualisation, methodology, data analysis, and manuscript drafting.

AAA: Laboratory investigation, data curation, visualisation, and critical revision of the manuscript.

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