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Molecular Menace: Dual Role of Resistance and Virulence in *Pseudomonas aeruginosa*

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a major nosocomial pathogen, especially in immunocompromised patients, due to its multidrug resistance and diverse virulence factors. Understanding the interplay between resistance and virulence is essential for effective management. **Methods:** A total of 195 non-duplicate *P. aeruginosa* isolates from blood, urine, respiratory, and exudative specimens were collected between August 2024 and February 2025 at a 1600-bedded university hospital. Identification was done using biochemical tests or VITEK®MS MALDI-TOF. Antimicrobial susceptibility testing was done following CLSI 2024 guidelines. PCR was used to detect virulence genes (*algD, aprA, plcH, lasB, exoS*) and carbapenemase genes (bla_{VIM}, bla_{IMP}, bla_{NDM}). **Results:** High resistance was observed to ciprofloxacin (n = 68), ceftazidime (n = 64), and carbapenems (n = 52). Among the carbapenem-resistant P. aeruginosa (CRPA) isolates, 17 harbored MBL genes (NDM = 6, IMP = 1, VIM = 1). Virulence genes were detected in 142 isolates, most commonly plcH (n = 92), lasB (n = 89), and algD (n = 71). Among the CRPA isolates, 73.1% carried at least one virulence gene. A significant association with carbapenem resistance was observed only for exoS ($p < 0.05$). **Conclusion:** This study highlights the need for surveillance of antimicrobial resistance and virulence factors in Pseudomonas aeruginosa. The co-expression of carbapenem resistance and virulence genes may contribute to the persistence of the pathogen and complicate treatment outcomes. Routine molecular profiling can support targeted therapeutic decisions and strengthen infection control measures. Further multicentric and genomic studies are warranted to elucidate the genetic interplay between resistance and virulence, thereby guiding effective antimicrobial stewardship strategies.

INTRODUCTION:

Pseudomonas aeruginosa is one of the most important causes of nosocomial infections, especially in immunocompromised patients. According to WHO, it is considered a critical priority pathogen due to its multidrug resistance and high mortality rates. The bacteria cause infections like UTIs, bloodstream infections, lung infections in cystic fibrosis, wound infections, and otitis media. The virulence factors that enhance tissue damage and resistance are pyocyanin, hemolysin, biofilms, and many enzymes. It evades treatment through multidrug efflux pumps, β-

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lactamases, and aminoglycoside-modifying enzymes, making infections difficult to manage.[1]

Carbapenemases are β-lactamases that are encoded by both chromosomal and plasmid-mediated genes. They are members of the A, B, and D Ambler classes. These potent β-lactamases hydrolyze a wide range of β-lactams, such as carbapenems, cephalosporins, penicillin, and aztreonam.[7] Carbapenem resistance in *P. aeruginosa* arises from a combination of β-lactamases (especially AmpC), porin mutations, MexA-MexB-OprM efflux pump overexpression, and/or penicillin-binding protein alterations. Combinations of mechanisms confer reduced susceptibility to carbapenems, but other β-lactam agents and aminoglycosides may retain *in vitro* activity. [6] So far, the most effective carbapenemases, in terms of carbapenem hydrolysis and geographical spread, are KPC, OXA-48, and the MBLs VIM, IMP, and NDM[7].

Pathogenicity of P. aeruginosa is attributed to the presence of various virulence factors namely: ***algD*** is crucial for alginate biosynthesis and biofilm formation, helping *P.aeruginosa* evade immune responses and antibiotics in cystic fibrosis patients, ***exoS*** gene encodes Exoenzyme S, which disrupts host cell signaling and cytoskeletal integrity[2], ***plcH*** gene encodes hemolytic phospholipase C, both of which damage host cell membranes, causing cell lysis and facilitating tissue invasion[3], ***lasB*** gene produces elastase promoting tissue damage and nutrient acquisition while evading immune responses[4], ***aprA*** gene encodes alkaline protease, which degrades immune proteins and cytokines, weakening the host's defenses and enhancing P. aeruginosa survival[5] and the other virulence encoding genes include: ***toxA, rhlAB, fliC, phzM, nan1***, etc.

The objectives of the current study are: (1) to characterise the virulence factors in clinical isolates of *Pseudomonas aeruginosa*, (2) to determine the susceptibility to various classes of antimicrobial agents, (3) to detect the carbapenemases by genotypic methods and (4) to study the association between virulence factors and antimicrobial susceptibility profile.

MATERIALS AND METHODS:

Bacterial strains:

The study was conducted in a 1600-bedded university teaching hospital from August 2024 – February. It included 195 clinically significant, non-duplicate, isolates of *Pseudomonas aeruginosa* acquired from clinical specimens of hospitalised patients. The isolates were obtained from clinical specimens such as blood, tissue, pus, wound swabs, and urine and lower respiratory secretions (bronchoalveolar lavage, bronchial wash and endotracheal secretions). Species identification utilized conventional biochemical methods and/or automated systems, including the VITEK® 2 GN card and MALDI-TOF MS (bioMérieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was determined by Kirby-Bauer disk diffusion and/VITEK 2 MIC, interpreted using CLSI 2024 guidelines. Virulence genes like *algD, aprA, plcH, lasB and exoS* and carbapenem resistance genes like NDM, IMP and VIM were identified using PCR.Care was taken to differentiate commensals from pathogens for isolates obtained from nonsterile sites (respiratory tract, urinary tract, and wound swabs). The significance of the isolates was based on clinical history, presence of the organism in the Gram stain, presence of intracellular forms of the organism, and pure growth in culture with a significant colony count.

Antimicrobial susceptibility testing :

Susceptibility to various classes of antibiotics was determined by the disc diffusion method in accordance with Clinical Laboratory Standard Institute (CLSI) guidelines. The antibiotics tested were amikacin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), piperacillin– tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg) and polymyxin B (300 units), from Himedia Laboratories (Mumbai, Maharashtra, India).

DNA extraction:

The DNA of the collected isolates were extracted using boiling and lysis procedure. The suspension of individual colonies in 400µl of TE buffer or distilled water. It was then boiled at 95°C for 10 mins and immediately freezed at -20°C for another 10 minutes, this was then centrifuged at 12000 rpm for 10 minutes. The suspension was collected and the pellet was discarded. 2µl of this suspension was used as template for amplification. The sequence was stored at -20°C for further analysis[13].

Detection of carbapenem resistance genes:

PCR for metallo-beta-lactamase genes: bla_{VIM}, bla_{IMP} and bla_{NDM} primers used are given in **Table 1.**

PCR conditions included 30 cycles of amplification under the following conditions: For VIM and IMP, denaturation at 95°C for 30 s, annealing for 1 min at specific temperatures (bla_{VIM}, 66°C, and bla_{IMP}, 45°C), and extension at 72°C for 1 min/kb product. Cycling was followed by a final extension at 72°C for 10 min.

For NDM, denaturation for 95°C for 5 min, 94°C for 30s, following by annealing at 55°C for 1 min,

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extension at 72°C for 1 min followed by 72°C for 5 min The PCR product of 500 bp (bla_{VIM}), 432 bp (bla_{IMP}) and bp (bla_{NDM}) was visualized by agarose gel electrophoresis.

Table 1: Primers used for detecting Carbapenem resistance genes

Primers	Amplicon size (bp)	References
bla_{IMP} Family- F GTTTATGTTCATACWTCG bla_{IMP} Family- R GGTTTAAYAAAACAACCAC	432 bp	(8)
bla_{VIM} Family- F TTTGGTCGCATATCGCAACG bla_{VIM} Family- R CCATTCAGCCAGATCGGCAT	500 bp	
bla_{NDM-1} Family- F GGG CAG TCG CTT CCA ACG GT bla_{NDM-1} Family- R GTA GTG CTC AGT GTC GGC AT	475 bp	(9)

Detection of virulence encoding genes:
PCR for virulence encoding genes: primers used are given in **Table 2.**

PCR conditions:
***lasB, plcH* and *exoS*:** Initial denaturation (94 °C for 5 min), followed by 25–30 cycles of denaturation (94 °C for 35–45 s), annealing (53– 62 °C from 45 s to 1 min), and extension (72 °C from 45 s to 1 min), with a single final extension at 72 °C for 7 min[10].

***algD*:** Initial denaturation step of two minutes at 95°C, followed by 35 cycles of denaturation (one minute at 95°C), annealing (30 seconds at 55°C) and extension (one minute at72°C); the reactions were finalized by polymerization for five minutes at 72°C[11].

***aprA*:** PCR programming was done with an initial denaturation at 95 °C for 5 min, followed by denaturation, annealing, and extension at 94 °C for 30 sec, 58 °C for 30 sec and 72 °C for 30 sec for 35 cycles. The final extension is at 72 °C for 5 min[12]

Table 2: Primers used for detection of Virulence encoding genes

Primers	Amplicon size(bp)	References
algD F: ATGCGAATCAGCATCTTTGGT *algD* R: CTACCAGCAGATGCCCTCGGC	520	(10,11,12)
aprA F: GTGCTGACCCTGTCCTATTC *aprA* R: GTGTTCTGCTCTTCCCAGTAG	506	
lasB F: GGAATGAACGAGGCGTTCTC *lasB* R: GGTCCAGTAGTAGCGGTTGG	300	
plcH F: GCACGTGGTCATCCTGATGC *plcH* R: TCCGTAGGCGTCGACGTAC	608	
exoS F: CTTGAAGGGACTCGACAAGG *exoS* R: TTCAGGTCCGCGTAGTGAAT	504	

DNA sequencing:
Representative isolates were subjected to automated DNA sequencing. Analysis of the DNA sequences were done using bioedit software. Similarity checks were performed using Nucleotide blast (blastN) (http://www.ncbi.nlm.nih.gov) and the sequences were submitted to GenBank and accession numbers were obtained.

RESULTS:

Out of the total 195 patients, 137 were male and 58 were females, and the age-wise distribution of the isolates was as follows: <20 years – 15/195 (7.7%), 20–40 years – 29/195 (14.9%), 41–60 years – 80/195 (41.0%), and >60 years – 71/195 (36.4%).

Among the total isolates (n = 195), 11 were from blood, 72 from urine, 24 from respiratory secretions, and 88 from exudative specimens.

The distribution of resistant isolates across various specimen types is presented in Table 3. Resistance to imipenem was observed in 26.2% of the isolates (51/195). Resistance to colistin was identified in 5 isolates, of which 4 were from urine and 1 from a respiratory sample.

Table 3: Antimicrobial profiling of the isolates according to the sample type

Antimicrobial drugs	Blood	Urine	Respiratory samples	Exudates	Total no. of resistant isolates
Piperacillin- tazobactam	1	33	2	18	54
Ceftazidime	1	34	3	25	63
Cefepime	1	33	1	20	55
Amikacin	0	32	1	13	46
Ciprofloxacin	1	38	6	22	67
Imipenem	1	33	2	15	51
Colistin	0	4	1	0	5

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Carbapenem resistance was detected using the disc diffusion method, identifying 51 isolates as carbapenem-resistant and 144 as carbapenem-sensitive. Detection of metallo-β-lactamase (MBL)-encoding genes was performed by PCR, and results were analyzed using gel electrophoresis. *bla*$_{NDM}$ was the most frequently identified, present in 20 isolates (10.2%). *bla*$_{IMP}$ was detected in 3 isolates (1.5%), while *bla*$_{VIM}$ was found in 2 isolates (1.03%). Among the 51 carbapenem-resistant P. aeruginosa (CRPA) isolates, 17 (8.7%) harbored MBL genes included in the study protocol.

Among the 51 imipenem-resistant isolates, 34 (68.8%) had at least one of the tested resistance genes. However, 17 imipenem-resistant isolates did not show amplification for *bla*$_{NDM,}$ *bla*$_{VIM}$, or *bla*$_{IMP.}$

Distribution of the Carbapenemase positive isolates among different clinical specimens is depicted in **Figure 1**

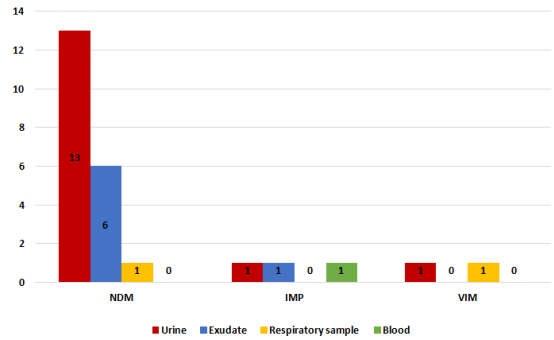

Figure 1: Distribution of Carbapenemase genes

In detection of virulence encoding genes, among the 195 isolates, 142(78.2%) carried one or more virulence genes. The most common virulence gene detected was *plcH* (n=92), followed by *lasB* (n=89), *algD* (n=71), *aprA* (n=57), and *exoS* (n=48). Out of the 51 CRPA isolates, 38 carried at least 1 virulence gene. Of the 142 isolates which carried the virulence genes, 20.5%(40 /195) carried a single gene and 102 harboured a combination of 2 or more genes. The most common 2 genes combination were: *algD* and *plcH* (n=54), followed by *lasB* and *plcH* (n=52), and *lasB* and *algD* (n= 48). The most common 3- gene combination was *algD+lasB+plcH* (n=38). The most common 4-gene combination was *algD+aprA+lasB+plcH* (n=10). All the gene combinations obtained in the study are mentioned in **Table 4.**

All 5 virulence genes were detected in 13 isolates.

Table 4: Gene Combinations and their percentages

Gene combinations	Number of isolates	Percentage
Two- gene combinations		
algD + plcH	54	27.7%
lasB + plcH	52	26.7%
lasB + algD	48	24.6%
exoS + lasB	45	23.1%
aprA+algD	40	20.5%
lasB+aprA	40	20.5%
plcH+aprA	36	18.5%
algD+exoS	29	14.9%
plcH+exoS	27	13.8%
aprA+exoS	24	12.3%
Three- gene combinations		
algD+aprA+lasB	31	15.9%
algD+aprA+exoS	19	9.4%
algD+aprA+plcH	29	14.9%
algD+lasB+exoS	27	13.8%
algD+lasB+plcH	38	19.5%
algD+exoS+plcH	22	11.3%
aprA+lasB+exoS	22	11.3%
aprA+lasB+plcH	27	13.8%
aprA+exoS+plcH	15	7.7%
lasB+exoS+plcH	26	13.3%
Four- gene combinations		
algD+aprA+lasB exoS	5	2.6%
algD+aprA+lasB+plcH	10	5.1%
algD+plcH+lasB+exoS	8	4.1%
aprA+lasB+exoS+plcH	1	0.5%
aprA+algD+exoS+plcH	1	0.5%

In relation to antibiotic resistance and the presence of virulence genes, a number of virulence genes were frequently observed among antibiotic-resistant isolates. The *lasB* gene was detected in 52.7% isolates resistant to piperacillin-tazobactam, 47.1% ciprofloxacin-resistant isolates, and 50% ceftazidime-resistant isolates. *plcH* gene was identified in 54.5% piperacillin-tazobactam-resistant isolates, 48.5% ciprofloxacin-resistant isolates, and 51.6% ceftazidime-resistant isolates. The *algD* gene was present in 47.3% piperacillin-tazobactam-resistant strains, 39.7% ciprofloxacin-resistant strains, and 42.2% ceftazidime-resistant strains.

The *aprA* gene was detected in 35.3% ciprofloxacin-resistant and 35.9% ceftazidime-resistant isolates, showing a relatively consistent distribution across these drug resistance profiles.In contrast, the *exoS* gene was detected in only 11.8% ciprofloxacin-resistant isolates 11.5% imipenem-resistant isolates, suggesting a lower prevalence of this gene among resistant strains.

Table 5 below show the distribution of virulence genes among the antimicrobial resistant isolates to each drug in this study.

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Table 5: Association of Antibiotic resistance with the presence of virulence genes

Resistant drug	No. of isolates	*lasB*	*plcH*	*algD*	*exoS*	*aprA*
Piperacillin-tazobactam	55	29	30	26	8	23
Cefepime	56	29	30	24	8	21
Ceftazidime	64	32	33	27	10	23
Imipenem	52	26	25	21	6	18
Colistin	5	2	2	3	1	2
Ciprofloxacin	68	32	33	27	8	24
Amikacin	47	26	24	21	7	20

Distribution of virulence genes among the carbapenem susceptible and resistant isolates are shown in table 6 . *p*- value was calculated with significance level at 0.05. Only *exoS* showed a statistically significant association with carbapenem resistance ($p< 0.05$). The other genes don't show a significant difference in distribution between susceptible and resistant groups.

Table 6: Distribution of virulence genes among the carbapenem susceptible and resistant isolates

Virulence gene	No. of isolates	Carbapenem susceptible (144)	%	Carbapenem resistant (51)	%	P value
algD	71	50	34.97	21	40.38	0.410412
lasB	89	63	44.06	26	50	0.373017
plcH	92	67	46.85	25	48.07	0.759353
aprA	57	39	27.27	18	34.62	0.267915
exoS	48	42	29.37	6	11.54	0.01317

DNA sequencing:

PCR positive amplicons for each virulence genes were purified and sequenced strains for each virulence gene served as positive controls. Sequencing was done by using Sanger AB 13730 XL DNA analyzing instrument(Immugenixbio, Tamil Nadu, India). Using Bio edit sequence programme(product version 7.0.5.3), the nucleotide sequences were aligned, and they were then compared with basic alignment search tool offered on National Centre for Biotechnology Information website (www.ncbi.nlm.nih.gov). The nucleotide sequences of these virulence genes were submitted to GenBank and the following accession numbers were obtained: PV021104, PV820565, PV820567, PV820566

DISCUSSION:

Overall, the most common source was exudative specimens, accounting for 45.1% of the total. Urine specimens accounted for 36.9%, followed by respiratory specimens at 12.3%, and blood at 5.6%. Majority of the patients were males(70.3%). The age of patients ranged from below 10 years to over 70, with the largest proportion (41%) belonging to the 41–60 age group. This predominance in middle-aged and older patients indicated a higher risk in adults with likely underlying health conditions or those receiving prolonged care. These findings closely align with external studies, such as Wahyunita et al.[20] where similar patient demographics and frequent sources like pus and wound swabs were observed contributing to 54.8% of the total isolates. The high recovery from wound and urinary sources was attributed to device-related infections and postoperative wound contamination in our study.

Antimicrobial Resistance Profile:

Antimicrobial susceptibility testing revealed variable resistance patterns. The highest resistance was observed to ciprofloxacin, with 67 isolates (34.4%) classified as resistant. Imipenem resistance was noted in 51 isolates (26.2%), while 63 isolates (32.3%) were resistant to ceftazidime. Resistance to cefepime and amikacin was recorded in 55 (28.2%) and 46 (23.6%) isolates, respectively. Piperacillin-tazobactam resistance was detected in 54 isolates (27.7%). Colistin susceptibility was assessed using broth microdilution, and all isolates except 5 showed MIC values within the susceptible range, indicating a low colistin resistance rate of 2.6%. These results reinforce colistin's continued therapeutic relevance.

Reyes et al.[14] reported significantly higher resistance in carbapenemase-producing CRPA isolates, with 94% ciprofloxacin resistance, 94% ceftazidime non-susceptibility, and 63% amikacin resistance. Ellappan et al.[15] also noted higher resistance, with 100% resistance to imipenem and meropenem, 97.4% to ciprofloxacin and ceftazidime, and 86.5% to amikacin. However, Rahman et al.[16] showed resistance rates that largely mirrored the current study's findings for imipenem (18.5%), ciprofloxacin (30.2%), ceftazidime (27.7%), piperacillin-tazobactam (17.9%), cefepime (19.1%), amikacin (24.6%), and colistin (3.4%)[17]. The presence of high resistance to first-line agents underscores the need for stringent antimicrobial stewardship and early diagnostic strategies.

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Carbapenem Resistance Gene Detection:
All 195 clinical isolates were tested for the presence of *bla*$_{NDM}$, *bla*$_{VIM}$, and *bla*$_{IMP}$ carbapenem resistance genes. *bla*$_{NDM}$ was the most frequently identified, present in 20 isolates (10.2%). *bla*$_{IMP}$ was detected in 3 isolates (1.5%), while *bla*$_{VIM}$ was found in 2 isolates (1.03%). No co-occurrence of carbapenemase genes was observed.

The majority of isolates with any of these genes were also phenotypically resistant to imipenem, confirming their contribution to resistance. Among the 51 imipenem-resistant isolates, 34 (68.8%) had at least one of the tested resistance genes. However, 17 imipenem-resistant isolates did not show amplification for *bla*$_{NDM}$, *bla*$_{VIM}$, or *bla*$_{IMP}$, suggesting alternative resistance mechanisms such as porin loss or efflux pump overexpression.

Comparative data showed variations. Amudhan et al.[21] reported *bla*$_{VIM}$ in 55.7% and *bla*$_{IMP}$ in 3.3% of their *P. aeruginosa* isolates but did not screen for *bla*$_{NDM}$. Reyes et al.[14] found carbapenemase genes in 22% of isolates globally, with *bla*$_{KPC}$ (49%) being the most common, followed by *bla*$_{VIM}$ (36%), *bla*$_{NDM}$ (7%), and *bla*$_{IMP}$ (6%). They also reported co-occurrence in 3% of isolates, which was not observed in the present study. Rahman et al.[16] reported a higher *bla*$_{NDM}$ rate (all CRPA isolates positive) in their specific cohort.

Virulence Gene Detection
All 195 isolates were screened for five key virulence genes: *algD*, *aprA*, *plcH*, *lasB*, and *exoS*. The detection rates varied: *plcH* was the most frequently identified (47.2%), followed by *lasB* (45.6%), *algD* (36.4%), *aprA* (29.2%), and *exoS* (24.6%). Only 20.5% (40/195) of isolates carried a single virulence gene, while 102 isolates (52.3%) carried more than one. A total of 64 isolates (32.8%) harbored three or more virulence genes.

The most frequent four-gene combination was *algD* + *aprA* + *plcH* + *lasB* (5.1%), followed by *algD* + *plcH* + *lasB* + *exoS* (4.1%). Only 13 isolates (6.7%) carried all five genes simultaneously. The high prevalence of multiple virulence genes indicated a broad pathogenic potential.

Comparisons with other studies showed variations in prevalence. Ghazaei et al.[17] found *lasB* in 68% of isolates, higher than our 45.6% . Rajabi et al. [18] detected *algD* in 77.5% of isolates, significantly higher than our 36.4%. Edward et al.[19] reported *exoS* in 63.3% and *lasB* in 55.0% of isolates, both higher than our observed rates. Despite these differences, the general patterns of virulence gene presence often aligned, supporting a conserved presence of these factors across clinical *P. aeruginosa* isolates.

Distribution of Virulence Genes in Clinical Specimens:
The distribution of virulence genes varied depending on the clinical specimen source.

- **Exudative specimens (n=88):** *plcH* was detected in 46.6%, *lasB* in 40.9%, *algD* in 27.3%, *aprA* in 20.5%, and *exoS* in 23.9%. The majority of exudative isolates carried three or more virulence genes.
- **Urinary isolates (n=72):** *plcH* was detected in 48.6%, *lasB* in 45.8%, *algD* in 44.4%, *aprA* in 38.9%, and *exoS* in 23.6%.'
- **Respiratory isolates (n=24):** *lasB* and *algD* were detected in 54.2% and 50% respectively. *plcH* and *aprA* were found in 45.3% and 33.3% respectively, while *exoS* was less frequent at 25%. The increased prevalence of *algD* suggested a role in biofilm formation and persistence in the airways.
- **Blood isolates (n=11):** Exhibited the lowest frequencies across most virulence genes, with *lasB* detected in 63.6%, *plcH* in 45.5%, and *exoS* in 36.4%. *algD* and *aprA* were present in 27.3% each.

Isolates from respiratory samples (83.3%) and urine (75%) carried a higher number of virulence determinants compared to those from exudates (67%) and blood (72.7%). This distribution suggests site-specific adaptations, with localized infections potentially necessitating a broader repertoire of virulence factors. These findings were moderately consistent with other studies on gene distribution across various clinical specimens.

Virulence Gene Patterns (Single vs. Combinations):
Among the 195 isolates, only 20.5% carried a single virulence gene, while 19.0% carried exactly two. A total of 32.8% harbored three or more virulence genes, indicating a broad arsenal of pathogenic factors. The most frequent 4-gene combination was *algD* + *aprA* + *plcH* + *lasB* (5.1%). *algD* and *plcH* were present in nearly 53% of multi-gene profiles, likely contributing to core pathogenic mechanisms. Isolates with four or more virulence genes were predominantly from exudative and urine samples. This complex and multifactorial nature of *P. aeruginosa* pathogenicity was supported by the presence of multiple virulence factors in most isolates. Comparisons to external studies, though sometimes limited by narrower gene panels, generally supported the trend toward multi-virulence carriage.

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Association Between Resistance and Virulence Genes:
A detailed comparison between antimicrobial resistance profiles and the presence of virulence genes revealed that carbapenemase-positive isolates also showed higher frequencies of multiple virulence genes. Among the 25 carbapenemase gene-positive isolates, 18 (72%) had at least three virulence genes. By contrast, among the 170 isolates without carbapenemase genes, only 45 (26.5%) carried three or more virulence determinants. Statistical analysis showed a weak but consistent association between resistance gene presence and high virulence gene load ($p < 0.05$). This suggests that certain *P. aeruginosa* strains circulating in the hospital environment might be equipped with both resistance and virulence traits, potentially increasing their capacity to cause severe or persistent infections.

Correlation with Clinical Features:
The clinical background of patients was analyzed in relation to resistance and virulence profiles. The majority of isolates were from patients admitted to intensive care units and surgical wards. The most common indications for culture were wound infections, respiratory complications, urinary tract infections, and bloodstream infections. Among isolates obtained from respiratory secretions, 41.7% carried three or more virulence genes, followed by urinary isolates (38.9%), blood isolates (36.4%), and exudates (25%). This suggests that patients with respiratory tract involvement were more likely to be infected with strains possessing both high virulence and antimicrobial resistance.

The combination of genetic data and patient factors indicated that certain infection sites, especially wounds and lungs, were more likely to yield *P. aeruginosa* isolates with enhanced pathogenic traits. This aligns with reviews highlighting the pathogen's prevalence in respiratory, urinary, and wound infections, and its capacity to form biofilms and produce virulence factors like elastase (*lasB*) in respiratory infections. While urinary tract infections are generally less virulent, the study's data showed that urinary isolates were second in the presence of multiple virulence factors and increased resistance. Edward et al.[19] further confirmed that respiratory tract and wound isolates commonly exhibited higher rates of both resistance and virulence factor presence.

CONCLUSION:
Among 195 *Pseudomonas aeruginosa* clinical isolates, key virulence genes such as *plcH* (47.2%), *lasB* (45.6%), and *algD* (36.4%) were frequently detected. Over 50% of isolates harbored two or more virulence genes, with notable combinations like *algD + aprA + plcH + lasB*, indicating a multifactorial virulence potential. The distribution varied by specimen type, with respiratory and urinary isolates showing higher multi-gene presence, supporting their clinical severity.

Antimicrobial resistance was highest to ciprofloxacin (34.4%) and ceftazidime (32.3%), with moderate resistance to piperacillin-tazobactam and imipenem. Colistin remained highly effective (97.4% susceptibility), reinforcing its role as a last-resort agent. Regional variability in resistance patterns emphasized the need for local antibiograms. Carbapenemase genes were identified in 12.8% of isolates: *blaNDM* (10.2%), *blaIMP* (1.5%), and *blaVIM* (1.03%). No co-occurrence of carbapenemase genes was observed, and some resistant isolates lacked these genes, suggesting alternative resistance mechanisms. A significant association was noted: 72% of carbapenemase-positive isolates carried ≥3 virulence genes compared to 26.5% among gene-negative isolates ($p < 0.05$). This correlation underscores the dual threat of antimicrobial resistance and enhanced pathogenicity in hospital-acquired strains.

Limitations:
The study had several limitations:
- Only five virulence genes (*algD, aprA, plcH, lasB, exoS*) were studied, which may not represent the complete spectrum of virulence determinants.
- The study investigated only three common carbapenem resistance genes (*blaNDM, blaVIM, blaIMP*), and other resistance mechanisms were not studied.
- The study did not assess the functional expression or phenotypic correlation of the detected genes, limiting the interpretation of their clinical impact.

Future Directions:
Future *P. aeruginosa* research should expand gene panels, including Whole-Genome Sequencing (WGS), to understand resistance and virulence. Studies on biofilm formation, clinical correlations, and vaccine development are crucial. Exploring alternative therapies like phage therapy and synergistic antibiotics is vital. Finally, integrating rapid diagnostics and combining local antibiogram data with virulence profiling will enhance antimicrobial stewardship and infection control.

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