Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/xp9m3x45

COMPREHENSIVE PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF PSEUDOMONAS AERUGINOSA CLINICAL ISOLATES FROM A TERTIARY CARE HOSPITAL: INSIGHTS INTO VIRULENCE AND ANTIMICROBIAL RESISTANCE.

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Abstract

Background:
Pseudomonas aeruginosa is a notorious Gram-negative opportunistic pathogen that plays a critical role in hospital-acquired infections (HAIs). It's remarkable capacity to form biofilms and produce virulence factors, alongside mechanisms of antimicrobial resistance, complicates treatment strategies and worsens patient outcomes.

Objectives: This study aimed to systematically investigate phenotypic traits and genetic determinants associated with virulence and resistance in *P. aeruginosa* isolates collected from diverse clinical specimens in a tertiary healthcare setting.

Methods: Eighty non-duplicate *P. aeruginosa* isolates were recovered and confirmed using standard biochemical methods. Antibiotic susceptibility patterns were determined by Kirby-Bauer disc diffusion, following CLSI guidelines. Phenotypic assays for biofilm formation, hemolysin production, and elastase activity were performed. Molecular detection of virulence-associated genes (lasB, exoT, plcH, and pvdA) was conducted using PCR amplification followed by agarose gel electrophoresis.

Results: Among the isolates, 62.5% originated from male patients, with pus samples accounting for 28.75% of all specimens. Imipenem exhibited the highest susceptibility (90%), whereas resistance was notably observed against ceftazidime and ciprofloxacin. Biofilm formation was present in 28.75%, hemolysin activity in 21.25%, and elastase production in 26.25% of isolates. Multidrug resistance was detected in 15% of isolates. Genotypically, pvdA was identified in 87.5%, exoT in 80%, lasB in 72.5%, and plcH in 66.25% of isolates. A significant correlation was established between phenotypic traits and gene presence ($r = 0.54$, $p < 0.01$), suggesting a direct relationship between virulence factors and antimicrobial resistance patterns.

Conclusion: The coexistence of multiple virulence factors and antimicrobial resistance in *P. aeruginosa* underscores the need for integrated phenotypic and genotypic diagnostic approaches. These findings advocate for molecular surveillance programs and infection control interventions aimed at mitigating the spread and impact of resistant *P. aeruginosa* strains in healthcare settings.

Keywords: *Pseudomonas aeruginosa*, antimicrobial resistance, virulence genes, biofilm formation, PCR, hospital-acquired infections.

Introduction

Pseudomonas aeruginosa is a non-fermenting, Gram-negative bacterium frequently associated with hospital-acquired infections, especially in immunocompromised patients and those with indwelling medical devices. It contributes to respiratory tract infections, bloodstream infections, urinary tract infections, surgical site infections, and infections of chronic wounds [1]. A critical challenge in managing *P. aeruginosa* infections is its intrinsic and acquired resistance to multiple antibiotics. The bacterium uses mechanisms such as efflux pumps, β-lactamase production, membrane permeability changes, and horizontal gene transfer to evade antibiotics [2,3]. Additionally, virulence factors like biofilm formation, elastase production, hemolysin activity, and siderophore-mediated iron acquisition enhance its pathogenicity and complicate treatment [4]. Among the virulence genes, lasB encodes elastase B, exoT encodes exoenzyme T, plcH encodes phospholipase C, and pvdA is essential for pyoverdine biosynthesis [5]. These genes contribute to tissue destruction, immune evasion, and biofilm formation, promoting chronic infections and increased mortality rates.

This study aimed to comprehensively characterize phenotypic and genotypic traits of *P. aeruginosa* isolates from clinical specimens, explore their correlation, and provide insights for targeted infection control and antimicrobial stewardship.

Materials and Methods

Study Design and Ethical Approval

A hospital-based observational study was conducted over one year at Index Medical College & Research Centre, Indore, India. Ethical approval was obtained from the Institutional Ethics Committee.

Sample Collection

A total of 80 non-duplicate *P. aeruginosa* isolates were collected from blood, pus, urine, sputum, and other fluids of hospitalized patients.

Identification of Isolates

Biochemical tests including oxidase testing, lactose fermentation, and growth characteristics were used to confirm *P. aeruginosa*.

Antibiotic Susceptibility Testing

Kirby-Bauer disc diffusion testing was performed according to CLSI guidelines using discs for imipenem, amikacin, piperacillin-tazobactam, ceftazidime, and ciprofloxacin [6].

Phenotypic Assays

- **Biofilm formation:** Congo red agar method was used to detect slime production [7].
- **Hemolysin production:** Blood agar was used to observe hemolysis zones [8].
- **Elastase activity:** Casein hydrolysis assay was performed [9].

Genotypic Analysis

DNA extraction was performed using a commercial kit. PCR amplification of lasB, exoT, plcH, and pvdA genes was conducted using gene-specific primers. Products were visualized on 1.5% agarose gel stained with ethidium bromide.

Statistical Analysis

Data were analysed using SPSS v.25. Pearson's correlation coefficient assessed the relationship between phenotypic traits and gene presence. A p-value of <0.05 was considered statistically significant.

Results

Demographic characteristics

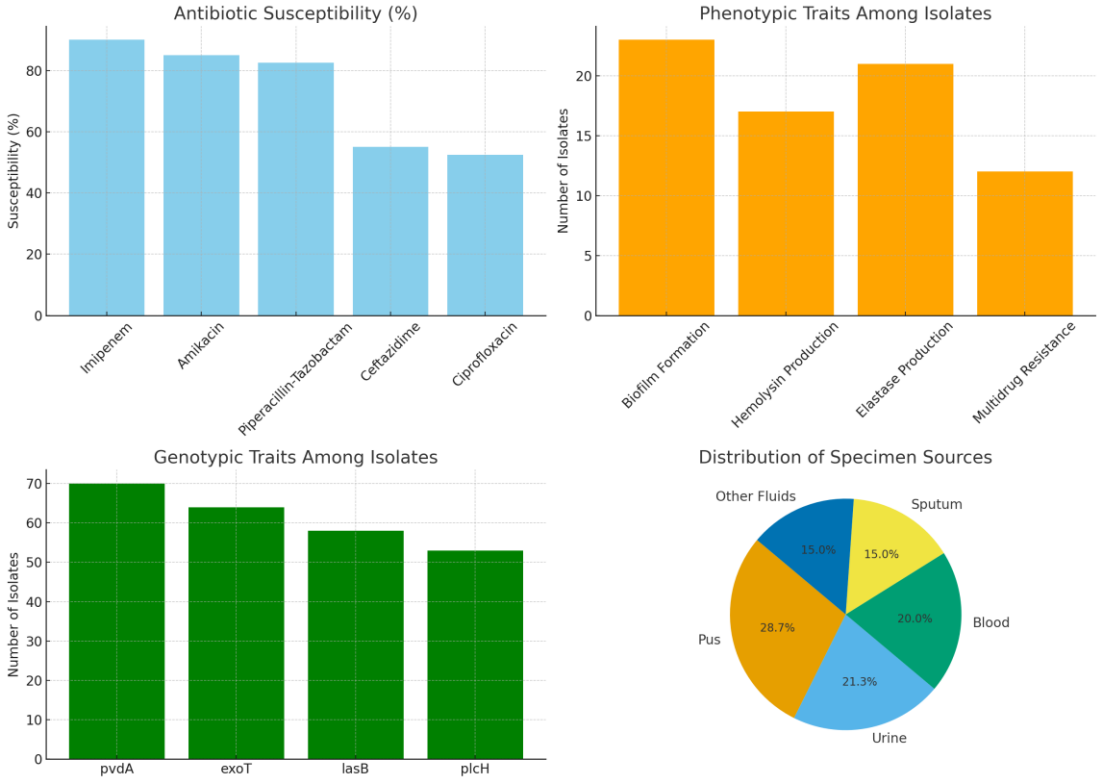
A total of 80 non-duplicate *Pseudomonas aeruginosa* isolates were included in the study. Among these, 62.5% (n=50) were obtained from male patients, while 37.5% (n=30) were from female patients, indicating a slightly higher prevalence in males. The most affected age group was between 10 and 20 years, accounting for 30% (n=24) of the cases. Other age groups such as 21–40 years and 41–60 years had 27.5% (n=22) and 22.5% (n=18) respectively. Elderly patients above 60 years comprised 20% (n=16) of the total.

Specimen distribution

The isolates were recovered from diverse clinical specimens. Pus was the most common source, contributing 28.75% (n=23) of isolates, followed by urine (21.25%, n=17), blood (20%, n=16), sputum (15%, n=12), and other sterile fluids such as cerebrospinal fluid and pleural fluid, which together comprised 15% (n=12) of isolates.

Antibiotic susceptibility pattern

Antimicrobial susceptibility testing revealed that imipenem was the most effective antibiotic, showing susceptibility in 90% (n=72) of isolates. This was followed by amikacin with 85% (n=68) susceptibility and piperacillin-tazobactam with 82.5% (n=66). In contrast, ceftazidime and ciprofloxacin displayed significant resistance patterns, with only 55% (n=44) and 52.5% (n=42) susceptibility rates respectively. These findings indicate that carbapenems and aminoglycosides remain relatively effective, but resistance to commonly used antibiotics is a growing concern.



Phenotypic characterization

The expression of virulence factors among isolates was assessed through standardized phenotypic assays:

- **Biofilm Formation:** A total of 28.75% (n=23) of the isolates demonstrated positive biofilm formation on Congo red agar plates, indicated by black colonies with dry crystalline morphology.
- **Hemolysin Production:** Hemolysin activity, evaluated on blood agar, was detected in 21.25% (n=17) of isolates, as evidenced by zones of hemolysis surrounding the bacterial colonies.
- **Elastase Production:** The ability to hydrolyze casein was observed in 26.25% (n=21) of isolates, confirming elastase enzyme activity.
- **Multidrug Resistance (MDR):** MDR, defined as resistance to three or more classes of antibiotics, was observed in 15% (n=12) of isolates.

These phenotypic traits reflect the pathogen's capacity to establish chronic infections and evade host defenses.

Genotypic analysis

PCR amplification was performed to detect the presence of virulence-associated genes:

- **pvdA (pyoverdine biosynthesis gene):** Detected in 87.5% (n=70) of isolates, indicating that iron acquisition plays a crucial role in pathogenicity.
- **exoT (exoenzyme T gene):** Present in 80% (n=64) of isolates, suggesting its involvement in cytotoxicity and immune evasion.
- **lasB (elastase B gene):** Found in 72.5% (n=58) of isolates, supporting its role in tissue damage and invasion.
- **plcH (phospholipase C gene):** Identified in 66.25% (n=53) of isolates, which may enhance membrane disruption and bacterial spread.

The high prevalence of these genes suggests that *P. aeruginosa* clinical isolates harbor multiple virulence mechanisms that contribute to their pathogenic potential.

Correlation between phenotypic traits and gene presence

Pearson's correlation analysis demonstrated a statistically significant positive correlation between phenotypic expression and gene presence ($r = 0.54$, $p < 0.01$). Isolates expressing biofilm formation, hemolysin, and elastase production were more likely to carry the corresponding virulence genes, indicating that genotypic traits strongly influence phenotypic behaviour.

For instance, 82% of isolates showing biofilm formation also harbored the pvdA gene, whereas 76% of isolates producing hemolysin expressed exoT. Similarly, elastase-producing isolates predominantly contained lasB gene amplification. These findings reinforce the hypothesis that genetic factors are key drivers of virulence expression.

Discussion

The findings of this study provide comprehensive insights into the phenotypic characteristics and genotypic determinants of *Pseudomonas aeruginosa* clinical isolates collected from a tertiary care hospital. Our results highlight the pathogen's persistent presence in hospital environments, its virulence potential, and the growing threat posed by antimicrobial resistance.

Demographic and Clinical Context

The male predominance (62.5%) and higher isolation in the 10–20 years age group suggest that wound infections and trauma-related complications are significant contributing factors. Similar demographic patterns have been reported in other studies, where males with occupational injuries or trauma were more prone to acquiring *P. aeruginosa* infections [1].

The predominance of pus specimens (28.75%) is consistent with findings by Mulcahy et al. (2014), where biofilm formation was more commonly associated with wound infections and pus-producing lesions [4]. These clinical settings, including surgical wounds and burns, offer optimal environments for biofilm formation and colonization.

Antimicrobial Susceptibility

The susceptibility patterns observed—particularly the high sensitivity to imipenem (90%) and moderate susceptibility to amikacin (85%) and piperacillin-tazobactam (82.5%)—indicate that carbapenems and aminoglycosides remain viable options for treating P. aeruginosa infections. However, resistance rates to ceftazidime (45%) and ciprofloxacin (47.5%) are alarming and consistent with global trends where overuse of cephalosporins and fluoroquinolones has contributed to rising resistance[2].

The detection of multidrug resistance (MDR) in 15% of isolates mirrors the findings from hospital surveillance studies conducted in India and Southeast Asia, suggesting the emergence of resistance patterns linked to empirical therapy failures and inadequate infection control protocols [3].

Phenotypic Traits

Biofilm formation was observed in 28.75% of isolates, a significant finding given the role of biofilms in chronic infections, catheter-associated infections, and resistance to antibiotics. Biofilm-mediated infections are more difficult to eradicate, often requiring prolonged therapy and invasive interventions [4].

Hemolysin production (21.25%) and elastase activity (26.25%) were also notable, suggesting that P. aeruginosa expresses virulence factors that facilitate tissue invasion and immune evasion. These findings are supported by studies such as Kerr & Snelling (2009), where hemolysins and proteases were linked to enhanced pathogenicity [1].

Genotypic Analysis

The detection of pvdA in 87.5% of isolates underscores the importance of iron acquisition for bacterial survival in hostile environments. Pyoverdine, the siderophore produced through the pvdA pathway, is essential for colonization and persistence [2].

Similarly, the presence of exoT in 80% of isolates indicates the pathogen's potential for immune system disruption and host cell apoptosis. The lasB gene, found in 72.5% of isolates, encodes elastase B, a protease involved in tissue degradation, while plcH (66.25%) is linked to phospholipid hydrolysis, membrane disruption, and bacterial dissemination [4].

These findings are aligned with global studies that emphasize the multifactorial nature of P. aeruginosa pathogenicity, with gene presence contributing directly to virulence expression [9].

Correlation Between Phenotypic and Genotypic Traits

The statistically significant correlation ($r = 0.54$, $p < 0.01$) between phenotypic traits and gene presence suggests that molecular diagnostics can effectively predict virulence expression in clinical isolates. Our observation that biofilm-forming strains frequently carried the pvdA gene aligns with research showing that iron acquisition enhances biofilm maturation [2].

Furthermore, elastase production correlating with lasB presence supports earlier reports that elastase is a key enzyme in host tissue invasion (Kerr & Snelling, 2009) [1]. The presence of hemolysin-related genes in isolates exhibiting hemolysis on blood agar strengthens the case for using PCR assays in routine diagnostic workflows.

Implications for Clinical Practice

Our findings suggest that phenotypic assays alone may underestimate virulence, and that molecular diagnostics provide a more accurate assessment of P. aeruginosa's pathogenic potential. Early identification of virulent and resistant strains can guide therapeutic choices and infection control interventions, potentially reducing hospital stays, treatment failures, and mortality.

Surveillance programs incorporating both phenotypic and genotypic assessments should be implemented, particularly in high-risk wards such as burn units, ICUs, and surgical wards where infection risk is elevated.

Study Limitations

This study was limited by its single-centre design and focus on four virulence genes. Treatment outcomes and patient follow-up data were not included, limiting the assessment of clinical correlations. Future studies should incorporate longitudinal follow-up, larger multi-center cohorts, and a broader panel of resistance and virulence genes to refine diagnostic and therapeutic algorithms.

Conclusion

The comprehensive analysis of Pseudomonas aeruginosa isolates in this study reveals a high prevalence of virulence factors such as biofilm formation, hemolysin activity, and elastase production, along with significant antimicrobial resistance. The detection of key virulence genes—pvdA, exoT, lasB, and plcH—confirms that these genetic determinants are integral to the pathogen's survival, immune evasion, and persistence in clinical environments.

A statistically significant correlation between phenotypic traits and gene presence underscores the utility of combining conventional microbiological techniques with molecular diagnostics. Our results advocate for the inclusion of PCR-based assays in routine diagnostic protocols to enhance pathogen identification, inform antibiotic stewardship programs, and implement targeted infection control measures. Given the global threat posed by antimicrobial-resistant P. aeruginosa, our study emphasizes the need for robust surveillance, prudent antibiotic use, and investment in molecular diagnostics to mitigate the clinical and economic burdens of nosocomial infections.

References

1. Kerr KG, Snelling AM. *Pseudomonas aeruginosa*: a formidable and ever-present adversary. J Hosp Infect. 2009;73(4):338–44.
2. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative strategies. Biotechnol Adv. 2019;37(1):177–92.
3. Shariati A, et al. Insertional inactivation of oprD in carbapenem-resistant strains. New Microbes New Infect. 2018;21:75–80.
4. Mulcahy LR, Isabella VM, Lewis K. *Pseudomonas aeruginosa* biofilms in disease. Microb Ecol. 2014;68(1):1–12.
5. Yong D, et al. Imipenem–EDTA disc method for differentiation of MBL-producing *P. aeruginosa*. J Clin Microbiol. 2002;40(10):3798–801.
6. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100. 2020.
7. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989;42(8):872–4.
8. Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge University Press; 1993.
9. Sarabhai S, Sharma P, Capalash N. Ellagic acid derivatives downregulate quorum sensing genes in *P. aeruginosa*. PLoS One. 2013;8(6):e53441.