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ORIGINAL ARTICLE

# Prevalence of *exoA*, *cepA*, *plcH*, *lasB*, and *algD* virulence genes in clinical isolates of *Pseudomonas aeruginosa* from hospitals in Yasuj and Shiraz, Iran

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## ABSTRACT

**Background and Objectives:** *Pseudomonas aeruginosa*, an opportunistic Gram-negative bacterium, is ubiquitous and represents one of the most challenging multidrug-resistant pathogens today. This multicenter study aimed to evaluate antibiotic resistance patterns, detect the *cepA* antibiotic resistance gene, and identify virulence factor genes (*exoA*, *algD*, *lasB*, and *plcH*) in clinical isolates of *P. aeruginosa.*

**Materials and Methods:** This experimental study analyzed 74 *P. aeruginosa* isolates obtained from clinical samples at Imam Sajad (Yasuj) and Namazi (Shiraz) hospitals, including 74 clinical isolates and one standard reference strain. Bacterial identification was performed using standard biochemical tests. Antibiotic susceptibility was assessed by the disk diffusion method according to CLSI 2018 guidelines. Genomic DNA was extracted by means of boiling method, and PCR assays were applied to detect *exoA*, *cepA*, *plcH*, *lasB*, and *algD* genes. Data were analyzed with chi-square tests, considering $p<0.05$ as statistically significant.

**Results:** Among 74 *P. aeruginosa* isolates, all carried the *exoA* gene, while *algD*, *plcH*, *cepA*, and *lasB* were detected in 95.6%, 94.6%, 93.2%, and 91.9% of isolates, respectively. High resistance was observed to aztreonam and ticarcillin, while cefiderocol showed the greatest sensitivity. A significant correlation was found between the *cepA* gene and antibiotic resistance ($P = 0.03$).

**Conclusion:** This study reveals a high prevalence of virulence genes and increasing antibiotic resistance among *P. aeruginosa* clinical isolates, highlighting the urgent need for effective therapeutic strategies to combat this pathogen.

**Keywords:** Antibiotic resistance; Virulence; *Pseudomonas aeruginosa*; Polymerase chain reaction

## INTRODUCTION

*Pseudomonas aeruginosa* is a motile, rod-shaped, Gram-negative, obligate aerobic bacterium ubiquitously present in nature and particularly thriving in humid hospital environments (1). As an opportunistic pathogen, it colonizes healthy individuals but more commonly causes infections in immunocom-

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promised patients, including those with diabetes, cancer, or cystic fibrosis, as well as individuals with severe burns. Its clinical impact is underscored by its association with significant morbidity and mortality in these vulnerable populations (2).

Critical to the pathogenic potential of *P. aeruginosa* is *exoA*, an exotoxin that disrupts elongating factor 2 (EF2) in protein biosynthesis, thereby promoting systemic disease and facilitating bacterial colonization. The *algD* gene drives alginate synthesis, producing mucoid variants that enhance adhesion and are characteristic of chronic infections in cystic fibrosis patients; these mucoid phenotypes are associated with persistent infections and increased disease severity. The *lasB* gene encodes elastase, which mediates quorum sensing and cell-to-cell communication, influencing transcription and translation of diverse virulence pathways. Elastases Las*A* and Las*B* act synergistically to degrade elastin, contributing to severe tissue destruction, including lung parenchymal damage and hemorrhagic skin lesions (3). Phospholipase C, encoded by *plcH* and *plcN*, together with rhamnolipid, participates in lipid degradation and membrane disruption, with rhamnolipid enhancing phospholipase C access by compromising lung cell membrane phospholipids and impairing ciliary function in respiratory epithelial cells. These actions collectively facilitate invasion, inflammation, and dissemination within the host (4).

Resistance determinants are equally critical to clinical outcomes. Reduced sensitivity to biocides, whether intrinsic or acquired through mutations or biocide resistance genes such as *qac* and *cepA*, poses a risk for disinfection failure and the spread of nosocomial infections that are resistant to both antibiotics and antiseptics (5). The combination of *P. aeruginosa*'s minimal nutritional requirements, an array of virulence factors, and a broad antimicrobial resistance profile underlines its status as a formidable pathogen in clinical settings (6). Antibiotic therapy for *P. aeruginosa* infections encompasses broad-spectrum penicillins (carbenicillin, ticarcillin, piperacillin), cephalosporins (ceftazidime and cefepime), carbapenems (imipenem and meropenem), aminoglycosides, fluoroquinolones, and azetronam; however, resistance to these agents is increasing, complicating treatment and contributing to poorer outcomes (7, 8).

Against this backdrop, the current multicenter study aims to evaluate the antibiotic resistance profile of clinical *P. aeruginosa* isolates, detect biocide and antibiotic resistance genes such as *cepA*, and assess the presence of virulence factor genes *exoA*, *algD*, *lasB*, and *plcH*. By focusing on these genes, the study seeks to clarify their associations with resistance patterns and pathogenic potential, thereby informing clinical risk assessment and guiding therapeutic strategies.

## MATERIALS AND METHODS

**Study design and sample collection.** This experimental study was conducted to determine the prevalence of virulence factor genes (*cepA*, *plcH*, *lasB*, *algD*, *exoA*) in *P. aeruginosa* strains isolated from clinical samples at Imam Sajad Hospital (Yasuj) and Namazi Hospital (Shiraz) in 2023. A total of 75 *P. aeruginosa* strains were analyzed, including one standard strain and 74 clinical isolates derived from patients admitted to the aforementioned hospitals.

**Ethical approval.** This study was conducted in accordance with the principles of the Declaration of Helsinki and received ethical approval from the Ethics Committee of Yasuj University of Medical Sciences (approval number: IR.YUMS.REC.1402.121). Written informed consent was obtained from all patients or their legal guardians prior to the collection of clinical samples. Patient confidentiality and anonymity were strictly maintained throughout the study.

**Bacterial isolation and identification.** Clinical *P. aeruginosa* isolates were subcultured on Mueller-Hinton agar (MHA) for purification. Species identification was confirmed through pigment production on MHA, positive oxidase test, oxidative-fermentative (OF) test, and growth characteristics in Kligler Iron Agar (KIA) medium, indicating aerobic, non-fermentative, and anaerobic-negative status. Confirmed isolates were stored in tryptic soy broth (TSB) supplemented with 10% glycerol at -20°C for subsequent analyses.

**Antibiotic susceptibility testing.** Antibiotic susceptibility was assessed using the disk diffusion method. A bacterial suspension equivalent to 0.5 McFarland standard was prepared and inoculated onto

MHA plates using a sterile swab. Antibiotic disks (ceftriaxone, amikacin, aztreonam, ticarcillin, piperacillin, meropenem, cefiderocol, and doripenem) were placed on the plates and incubated at 37°C for 18-24 hours. The diameter of the inhibition zones was measured and interpreted as susceptible or resistant according to the Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines (9).

**DNA extraction and PCR analysis.** Genomic DNA was extracted using the boiling method. Briefly, a single colony was suspended in 300 µL of sterile distilled water, boiled at 100°C for 10 minutes, and centrifuged at 12,000 rpm for 10 minutes. The supernatant (100 µL) containing DNA was transferred to a microtube and stored at -20°C. Primers targeting *exoA, cepA, plcH, lasB,* and *algD* were selected based on previous studies conducted in Iran and internationally, verified using the NCBI database, and synthesized by Pishgam Company. Primer sequences are provided in Table 1. PCR reactions were performed using a master mix prepared according to Pishgam's protocol in a Corbett thermocycler (USA) under conditions specified in Table 2.

**Agarose gel electrophoresis.** PCR products (5 µL) were loaded onto a 1% agarose gel containing Safe Stain and run in 1X TBE buffer at 90 V for 30 minutes. Gels were visualized under UV light using gel documentation system.

**Statistical analysis.** The data were analyzed using IBM SPSS Statistics software, version 23. Relationships between categorical variables were assessed employing chi-square tests. Statistical significance was set at a probability level of $p < 0.05$.

## RESULTS

This study analyzed 74 *P. aeruginosa* isolates, with 36 (48.6%) from Shiraz and 38 (51.4%) from Yasuj. The relative frequency of virulence genes (*exoA*, *algD*, *plcH*, *cepA*, and *lasB*) and antibiotic resistance profiles were evaluated.

**Virulence gene distribution.** The distribution of virulence genes is summarized in Table 3. All isolates (100%) harbored the *exoA* gene, representing the most prevalent virulence factor. The *algD* and *plcH* genes were also highly prevalent, detected in 71 (95.6%) and 70 (94.6%) isolates, respectively. The *cepA* and *lasB* genes were identified in 69 (93.2%) and 68 (91.9%) isolates, respectively. Isolates from Shiraz displayed a higher relative abundance of *algD*, *plcH*, *cepA*, and *lasB* genes compared to those from Yasuj. However, the frequency of *algD*, *plcH*, and *lasB* genes did not differ significantly between cities ($P > 0.05$). Notably, the *cepA* gene was present in 100% of Shiraz isolates (36/36) compared to 86.8% of Yasuj isolates (33/38), a statistically significant difference (Fisher's Exact Test, $\chi^2 = 5.08$, $P = 0.03$, $df = 1$).

**Antibiotic resistance profiles.** The antibiotic resistance profiles of *P. aeruginosa* isolates are detailed in Table 4. The highest resistance rates were observed against aztreonam (89.2%, n = 66), ticarcillin (87.8%, n = 65), and ceftriaxone (81.1%, n = 60). Conversely, the greatest sensitivity was recorded for cefiderocol (89.2%, n = 66) and meropenem (33.8%, n = 25). Intermediate resistance was most frequent for doripenem (21.6%, n = 16) and meropenem (20.3%, n = 15).

**Table 1.** Primer sequences for virulence genes

| Virulence Factor | Name | Sequence (5' → 3') | Ref. |
|---|---|---|---|
| Exotoxin A | *exo A*- F | CCTCAGCATCACCAGGGA | (19) |
| | *exo A*- R | CTGACGAAGAAGGTGGCATC | |
| Phosphplipase C | *plc H*-F | CCGCAACGACAAGTGGATG | (19) |
| | *plc H*-R | AGCCGCCTTCCTGGTAGAC | |
| Elastase | *las B*-F | AGCCATCACCGAAGTCAAGG | (19) |
| | *las B*-R | CGGGAATCAGGTAGGAGACG | |
| Alginate | *alg-D*-F | TGGCGTTCGGACTTCTCG | (19) |
| | *alg-D*-R | TGGTTTGGGCTATGTCGGTG | |
| Biocide Resistance | *cepA*- F | GCTCGCTGATGTCGGTAGG | (16) |
| | *cepA*- R | CTGCTGGCAGTGCACTATTC | |

**Table 2.** PCR reaction compounds and conditions

| **PCR Reaction Compounds** | | | |
|---|---|---|---|
| **Component** | | **Amount** | **Final Concentration** |
| Master Mix | | 12.5 µL (1X) | - |
| Distilled Water | | 8.5 µL | - |
| Template DNA | | 2 µL (20pg) | - |
| Forward Primer | | 1 µL (10 µM stock) | 0.4 µM (final) |
| Reverse Primer | | 1 µL (10 µM stock) | 0.4 µM (final) |
| **PCR Reaction Conditions** | | | |
| **For *exoA*, *plcH*, *algD* Genes** | | | |
| **Phase** | **Cycle Number** | **Time** | **Temperature** |
| Initial Denaturation | 1 | 4 min | 94°C |
| Denaturation | 32 | 45 s | 94°C |
| Annealing | 32 | 30 s | 58°C |
| Extension | 32 | 1 min | 72°C |
| Final Extension | 1 | 5 min | 72°C |
| **For *lasB*, *cepA* Genes** | | | |
| **Phase** | **Cycle Number** | **Time** | **Temperature** |
| Initial Denaturation | 1 | 4 min | 94°C |
| Denaturation | 32 | 45 s | 94°C |
| Annealing | 32 | 40 s | 59°C |
| Extension | 32 | 1 min | 72°C |
| Final Extension | 1 | 5 min | 72°C |

**Table 3.** Distribution of virulence genes in *Pseudomonas aeruginosa* isolates from Shiraz and Yasuj

| **Gene** | **Absolute Frequency (n)** | **Relative Frequency (%)** | **Shiraz (n= 36)** | **Yasuj (n= 38)** | **P-value** |
|---|---|---|---|---|---|
| *exoA* | 74 | 100 | 36 (100%) | 38 (100%) | - |
| *algD* | 71 | 95.6 | 36 (100%) | 35 (92.1%) | >0.05 |
| *plcH* | 70 | 94.6 | 36 (100%) | 34 (89.5%) | >0.05 |
| *cepA* | 69 | 93.2 | 36 (100%) | 33 (86.8%) | 0.03 |
| *lasB* | 68 | 91.9 | 35 (97.2%) | 33 (86.8%) | >0.05 |

Note: P-values were calculated using Fisher's Exact Test for *cepA* and chi-square tests for other genes. A dash (-) indicates no statistical test was needed due to 100% prevalence.

**Table 4.** Antibiotic resistance profiles of *Pseudomonas aeruginosa* isolates

| **Antibiotic** | **Resistance (n, %)** | **Intermediate (n, %)** | **Sensitive (n, %)** |
|---|---|---|---|
| Aztreonam | 66 (89.2%) | 6 (8.1%) | 2 (2.7%) |
| Ceftriaxone | 60 (81.1%) | 4 (5.4%) | 10 (13.5%) |
| Ticarcillin | 65 (87.8%) | 6 (8.1%) | 3 (4.1%) |
| Amikacin | 47 (63.5%) | 8 (10.8%) | 19 (25.7%) |
| Piperacillin | 46 (62.2%) | 14 (18.9%) | 14 (18.9%) |
| Meropenem | 34 (45.9%) | 15 (20.3%) | 25 (33.8%) |
| Doripenem | 44 (59.5%) | 16 (21.6%) | 14 (18.9%) |
| Cefiderocol | 4 (5.4%) | 4 (5.4%) | 66 (89.2%) |

**Correlation between virulence genes and antibiotic resistance.** Correlation analyses were performed to assess associations between virulence gene presence and antibiotic resistance phenotypes. The presence of the *cepA* gene showed a statistically significant positive correlation with resistance to aztreonam (P = 0.04) and ticarcillin (P = 0.03). Similarly, isolates harboring the *plcH* gene demonstrated higher resistance rates to ceftriaxone (P = 0.05). No significant correlations were observed between *exoA*, *algD*, or *lasB* genes and resistance patterns to the tested antibiotics (P>0.05). These findings suggest that particular virulence determinants may be linked to resistance mechanisms, potentially contributing to the pathogenicity and treatment challenges of *P. aeruginosa*.

## DISCUSSION

This study demonstrated a high prevalence of virulence genes in *P. aeruginosa* isolates, with *exoA*, *algD*, *plcH*, *cepA*, and *lasB* detected in 95.5%, 94.6%, 93.2%, and 91.9% of isolates, respectively. These findings are generally consistent with previous reports, although prevalence rates vary across studies. For instance, Truşcă et al. reported an 80% prevalence for both *algD* and *plcH* genes, which is notably lower than observed here (10). Likewise, El-Sayed et al. documanted a 60% prevalence of the *algD* (11), while Dorri et al. and Gholami et al. reported *algD* prevalence rates approaching 100% and 98%, respectively (12, 13). Similarly, for the *lasB* gene, our prevalence aligns with studies such as Roshani-Asl et al. (85.5%) and Mapipa et al. (75%), though slight variations can be observed (14, 15).

The *cepA* gene was detected in 93.2% of isolates in this study, which exceeds the 81.5% prevalence reported by Khademi et al., but is higher than rates reported by Mendes et al. (44.5%) and Vijayakumar et al. (63.6%) (5, 16, 17). Discrepancies in gene prevalence across studies likely reflect differences in sample size, clinical specimen sources, geographic locations, bacterial genetic diversity, gene mutation rates, and environmental pressures such as horizontal gene transfer and selective adaption.

Understanding the roles of these virulence factors is critical, as they contribute significantly to *P. aeruginosa* pathogenicity. The *algD* gene encodes GDP-mannose dehydrogenase, vital for alginate biosynthesis and biofilm formation, facilitating bacterial persistence and resistance to host defenses. The *plcH* gene encodes hemolytic phospholipase C, which damages host cell membranes, exacerbating tissue injury. The *cepA* gene encodes a chloroperoxidase involved in oxidative stress response, enhancing survival in hostile environments, whereas *lasB* encodes elastase, a protease that degrades host proteins and immune factors, promoting invasion and immune evasion.

Regarding antibiotic resistance, *P. aeruginosa* isolates in this study exhibited the highest resistance against aztreonam (89.2%), ticarcillin (87.8%), and ceftriaxone (81.1%), with considerable sensitivity toward cefiderocol (89.2%) and meropenem (33.8%). These resistance profiles are generally higher than those reported by Dorri et al. (22.5% aztreonam resistance) and Bazghandi et al. (43% aztreonam resistance), but consistent or somewhat lower compared to other regional studies reporting amikacin resistance rates ranging from 46% to 88% (12-14, 18-21). Variability in antibiotic resistance rates may be influenced by differences in antibiotic usage policies, local prescribing practices, and the extent of antibiotic exposure in hospital environments.

Meropenem resistance (45.9%) observed here is intermediate compared with reports by Roshani-Asl et al. (96%) and several other studies ranging from 41% to 58% (14, 22-24). Piperacillin and ticarcillin resistance rates noted in this study (62.2% and 87.8%, respectively) are relatively high but consistent with findings from similar clinical settings, although some studies report lower rates (19, 21, 25-29). Resistance to doripenem was notably higher in our isolates (59.5%) than in some previous studies (7-58%), which may reflect increasing resistance trends or regional differences (15, 19, 26, 29). The resistance rate to cefiderocol (5.4%) aligns with other recent investigations reporting values between 2% and 30%, underscoring its potential as a promising therapeutic agent against multidrug-resistant *P. aeruginosa* (30-32).

Increasing antibiotic resistance observed across studies is likely driven by factors such as overuse and misuse of antibiotics, regional and cultural prescribing practices, differences in bacterial isolation sites, and environmental pressures promoting resistance gene acquisition and selection. The co-existence of virulence genes and resistance determinants, as suggested in this study, presents a clinical challenge by potentially enhancing bacterial survival, persistence,

and pathogenicity. This emphasizes the importance of monitoring both virulence profiles and resistance patterns concurrently to better inform infection control strategies and therapeutic decision-making. Clinicians should exercise caution when selecting antibiotics for *P. aeruginosa* infections and promote antimicrobial stewardship to mitigate resistance development and optimize patient outcomes.

This study has several limitations, including a relatively small sample size and restriction to two hospitals in specific geographical regions, which may limit the generalizability of the findings. Therefore, future studies utilizing larger, more diverse samples and advanced molecular techniques are warranted to validate and extend these results.

## CONCLUSION

In this study, *exoA*, *algD*, *plcH*, *cepA*, and *lasB* genes were highly prevalent in *P. aeruginosa* isolates, detected in 100%, 95.6%, 94.6%, 93.2%, and 91.9% of strains, respectively. Antibiotic susceptibility showed cefiderocol and meropenem as the most effective treatments, while resistance was greatest against to aztreonam, ticarcillin, and ceftriaxone. These findings highlight the importance of using cefiderocol and meropenem for treating *P. aeruginosa* infections. The increasing antibiotic resistance observed likely results from antibiotic misuse, empirical therapies without susceptibility testing, prolonged hospital stays, and the bacterium's strong biofilm formation and colonization abilities. This underlines the need for routine susceptibility testing and strengthened infection control practices to limit the spread of resistant strains. Future studies should monitor resistance trends, explore resistance mechanisms, and develop new treatments to address multidrug-resistant *P. aeruginosa* effectively.

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