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Original Article

Expression of *bla$_{SHV}$* in Beta-Lactam-Resistant *Pseudomonas aeruginosa* Clinical Isolates from Mashhad

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Abstract

The role of *bla$_{SHV}$* expression in beta-lactam resistance among clinical isolates of *Pseudomonas aeruginosa* is not fully understood. This study aimed to investigate the expression level of the *bla$_{SHV}$* gene in beta-lactam-resistant *P. aeruginosa* clinical isolates obtained from hospitals in Mashhad. In this cross-sectional study, 103 clinical isolates of *P. aeruginosa* were recovered from different hospital wards and clinical specimens. Antimicrobial susceptibility testing was performed against several β-lactam antibiotics, including third- and fourth-generation cephalosporins. Based on beta-lactam resistance profiles, particularly resistance to cephalosporins, 20 representative isolates were selected for analysis of *bla$_{SHV}$* gene expression using real-time quantitative PCR (RT-qPCR). Total RNA was extracted, converted to cDNA, and gene expression levels were normalized to the *16S rRNA* reference gene. Relative expression was calculated using the $2^{-}\Delta\Delta Ct$ method with *P. aeruginosa* PAO1 as the calibrator strain. Statistical analysis was performed to compare expression levels with the reference strain. High resistance rates to several cephalosporins were observed among the isolates, including ceftriaxone (100%), ceftiofur (93.2%), ceftazidime (69%), and cefepime (68.2%). All analyzed isolates showed a significant upregulation of *bla$_{SHV}$* expression compared with the PAO1 reference strain ($p < 0.05$). Increased *bla$_{SHV}$* expression was also detected in some imipenem-susceptible isolates, suggesting that transcriptional upregulation of this gene is not directly associated with carbapenem resistance. Upregulation of *bla$_{SHV}$* is associated with resistance to extended-spectrum cephalosporins in beta-lactam-resistant *P. aeruginosa* clinical isolates from Mashhad. Further studies are required to clarify the clinical implications of *bla$_{SHV}$* overexpression and its contribution to beta-lactam resistance mechanisms.

Keywords: Antimicrobial resistance; Gene expression; Hospital infections; RT-qPCR

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Introduction

Antimicrobial resistance (AMR) represents a major global public health challenge, as the increasing prevalence of resistant Gram-negative pathogens continues to compromise effective therapeutic options in hospital settings. Among these or78ganisms, *P. aeruginosa* is of particular concern due to its intrinsic resistance mechanisms, remarkable genomic plasticity, and its capacity acquire and regulate additional resistance determinants under antibiotic selective pressure (1,2). *P. aeruginosa* is a common cause of healthcare-associated infections, including wound, burn, bloodstream, urinary tract, and respiratory tract infections, particularly among patients admitted to intensive care units (ICUs) and those with prolonged hospitalization or impaired immune function. Infections caused by antibiotic-resistant *P. aeruginosa* are associated with increased morbidity and mortality, prolonged hospital stays, and substantial healthcare costs, highlighting the importance of continuous epidemiological and molecular surveillance (3,5). β-Lactam antibiotics remain a cornerstone of therapy for *P. aeruginosa* infections. However, their clinical efficacy has increasingly been compromised by the production of β-lactamase enzymes. Extended-spectrum β-lactamases (ESBLs), particularly class A enzymes such as SHV-type β-lactamases, can hydrolyze penicillins and extended-spectrum cephalosporins, thereby reducing the effectiveness of these antibiotics (6). The bla_{SHV} gene has been reported in clinical isolates of *P. aeruginosa* from various geographical regions, including isolates obtained from burn and wound infections, indicating its potential role in hospital-associated infections (7,8). In this study, the presence of the bla_{SHV} gene was confirmed in all *P. aeruginosa* clinical isolates by conventional PCR prior to expression analysis, ensuring that the evaluated isolates indeed harbored this gene. Furthermore, regional data indicates a notable prevalence of bla_{SHV} in clinical isolates from hospital settings in Mashhad and similar geographic areas, corroborating the relevance of focusing on bla_{SHV} -mediated β-lactam resistance in this population (6,7). Although several studies have reported the molecular detection of bla_{SHV} in *P. aeruginosa*, most investigations have primarily focused on gene presence rather than transcriptional activity. Importantly, the presence of a resistance gene does not necessarily reflect its functional contribution to antimicrobial resistance, as the level of β-lactamase expression can significantly influence the phenotypic resistance profile (9). Furthermore, SHV-type β-lactamases are not considered major carbapenem-hydrolyzing enzymes. Carbapenem resistance in *P. aeruginosa* is more commonly associated with other mechanisms such as carbapenemase production, efflux pump overexpression, or alterations in outer membrane porins (2,10). Therefore, quantitative assessment of bla_{SHV} expression may provide valuable insight into its contribution to resistance against extended-spectrum cephalosporins rather than carbapenems. Accordingly, the present study aimed to evaluate the relative expression of the bla_{SHV} gene in clinical isolates of *P. aeruginosa* with reduced susceptibility to β-lactam antibiotics using quantitative real-time PCR (RT-qPCR). By normalizing gene expression levels to the *16S rRNA* housekeeping gene and comparing clinical isolates with the reference strain *P. aeruginosa* PAO1, this study provides molecular-level evidence regarding the contribution of bla_{SHV} expression to β-lactam resistance in hospital-associated *P. aeruginosa* infections.

Methods

Bacterial Isolates and Study Design

This cross-sectional study was conducted on clinical isolates of *P. aeruginosa* obtained from hospitalized patients in Mashhad, Iran. The isolates were collected from different wards of Imam Reza hospital during the period from May to October 2023. A total of 103 non-duplicate isolates were collected from various clinical specimens and hospital wards. Bacterial identification was confirmed using standard biochemical tests. Antimicrobial susceptibility testing was performed according to CLSI guidelines (11) for several beta-lactam antibiotics, including ceftazidime, cefepime, ceftriaxone, ceftiofur, and imipenem. Clinical isolates showing reduced susceptibility to one or more of these beta-lactam agents were included for further analysis. Identification of the bla_{SHV} gene in all isolates was initially confirmed using conventional PCR before proceeding to gene expression analysis. This ensured that only

isolates harboring the *bla*$_{SHV}$ gene were included in the expression study. Isolates were selected solely based on their reduced susceptibility to beta-lactam antibiotics, without applying multidrug resistance criteria.

For gene expression analysis, 20 representative beta-lactam-resistant isolates (including both imipenem-resistant and imipenem-susceptible strains) were selected. The relative expression level of the *bla*$_{SHV}$ gene was evaluated using RT-qPCR. The reference strain *P. aeruginosa* PAO1 was used as the calibrator for comparative expression analysis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the disk diffusion method on Mueller–Hinton agar (MHA; Ibresco Iran) according to CLSI guidelines (11). Fresh bacterial colonies were suspended in sterile saline to a 0.5 McFarland standard and inoculated onto MHA plates using the lawn culture technique.

RNA Extraction and Quality Assessment

Total RNA was extracted from overnight cultures of *P. aeruginosa* isolates using a silica column–based RNA extraction kit (Pars Tous , Iran) according to the manufacturer's instructions. Briefly, following cell lysis, 400 µL of the clarified lysate was transferred to a new tube and mixed with 400 µL of 70% ethanol. The mixture was applied to the spin column and centrifuged at 13,000 rpm for 1 min. After removal of the flow-through, the column was washed with 700 µL of peptone water buffer and centrifuged at 13,000 rpm for 1 min. An additional centrifugation step for 2 min was performed to ensure complete removal of residual wash buffer.

RNA was eluted by adding 50 µL of DEPC-treated nuclease-free water to the column, followed by incubation at room temperature for 3 min and centrifugation at 13,000 rpm for 1 min. The purified RNA samples were collected in RNase-free microtubes and stored at −70 °C until further analysis.

RNA concentration was determined by measuring absorbance at 260 nm, and RNA purity was assessed using the A260/A280 ratio. All measurements were performed using a NanoDrop spectrophotometer.

cDNA Synthesis

Prior to cDNA synthesis, RNA samples were normalized to minimize variability associated with differences in initial RNA concentrations. The sample with the lowest RNA concentration was identified, and the RNA input for all reactions was adjusted to ensure equal amounts of total RNA.

Complementary DNA (cDNA) was synthesized using a commercial reverse transcription kit (Pars Tous, Iran) according to the manufacturer's instructions. Each reaction contained 10 ng of normalized total RNA, 10 µL of 2× RT buffer, 2 µL of reverse transcriptase enzyme mix, and nuclease-free DEPC-treated water to a final volume of 20 µL.

Thermal conditions included primer annealing at 25 °C for 10 min, reverse transcription at 47 °C for 60 min, and enzyme inactivation at 85 °C for 5 min. The synthesized cDNA was immediately placed on ice and stored at −20 °C until further use.

Primer Preparation

Lyophilized primers specific for the *bla*$_{SHV}$ gene and the internal reference gene (*16S rRNA*) were reconstituted with sterile deionized water according to the manufacturer's instructions (Sinaclon, Iran). The stock primer solutions were subsequently diluted 1:10 to obtain working solutions with a final concentration of 10 µM for both forward and reverse primers. The primer sequences used in this study are presented in Table 1.

The primer sequences were provided by the manufacturer (Sinaclon, Iran), and their specificity was confirmed using NCBI BLAST analysis.

Table 1. Primer sequences used in this study.

Target gene	Primer sequence (5′–3′)
bla$_{SHV}$	F: GATGAACGCTTTCCCATGATG
	R: CGCTGTTATCGCTCATGGTAA
16S rRNA	F: CAGCTCGTGTCGTGAGATGT
	R: CGTAAGGGCCATGATGACTT

Primer Efficiency Validation and Replicates

Prior to the gene expression analysis, primer amplification efficiencies for *blaSHV* and *16S rRNA* were determined by generating standard curves using serial dilutions of cDNA. Both primer sets showed efficiencies within the acceptable range of 90–110%, confirming the suitability of the primers for relative quantification using the 2^-ΔΔCt method. All qPCR reactions were performed in triplicates as technical replicates to ensure reproducibility, and experiments were conducted on at least three independent biological samples (biological replicates).

RT-qPCR

RT-qPCR was performed using SYBR Green chemistry on a Rotor-Gene Q real-time PCR system. Each reaction was carried out in a final volume of 20 µL containing 10 µL of SYBR Green master mix, 2 µL of cDNA template, 1 µL of each forward and reverse primer (10 µM), and 6 µL of nuclease-free water.

The thermal cycling program included an initial denaturation at 95 °C for 1 min, followed by 35 We selected *16S rRNA* as the reference gene due to its widespread use in bacterial gene expression studies including *P.aeruginosa*. Although *16S rRNA* is highly expressed, its use can provide reliable normalization when primer efficiencies are validated. Amplification efficiencies of *bla*$_{SHV}$ and *16S rRNA* were confirmed to be comparable in this study. Nonetheless, alternative housekeeping genes such as *rpoD* or *gyrA* may also be considered in future studies.

Statistical Analysis

Data was analyzed using GraphPad Prism (v10.1.2). Relative *blaSHV* expression ($2^{-\Delta\Delta Ct}$) was compared to the reference PAO1 strain via one-way ANOVA followed by Dunnett's test. Statistical significance was defined as $P<0.05$.

cycles of denaturation at 95 °C for 30 s, primer annealing at 58 °C for 30 s, and extension at 72 °C for 40 s, with a final extension step at 72 °C for 10 min. A melting curve analysis was performed at the end of the amplification cycles to verify the specificity of PCR products and to confirm the absence of non-specific amplification.

Relative Gene Expression Analysis

Threshold cycle (Ct) values were automatically calculated for each reaction. Relative expression of the *bla*$_{SHV}$ gene was quantified using the comparative Ct (ΔΔCt) method. Gene expression levels were normalized to the internal reference gene *16S rRNA* and calculated relative to the control strain *P. aeruginosa* PAO1 using the equation:

Relative expression=$2^{-\Delta\Delta Ct}$

were

$$\Delta Ct = Ct_{target} - Ct_{reference}$$

and

$$\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{control}$$

Results

Distribution of *P. aeruginosa*

A total of 103 clinical isolates of *P. aeruginosa* were collected from different hospital wards and clinical specimens. The highest proportion of isolates was obtained from the ICU (27.2%), followed by the urology and internal medicine wards (each 16.5%). Infectious diseases and emergency wards each accounted for 5.8% of the isolates. Lower frequencies were observed in the burn unit, general surgery, coronary care unit (CCU), and pulmonary wards. Additional isolates were obtained from other hospital units, including neurology, orthopedics, otorhinolaryngology, and central operating rooms, each contributing approximately 1–2% of the total isolates.

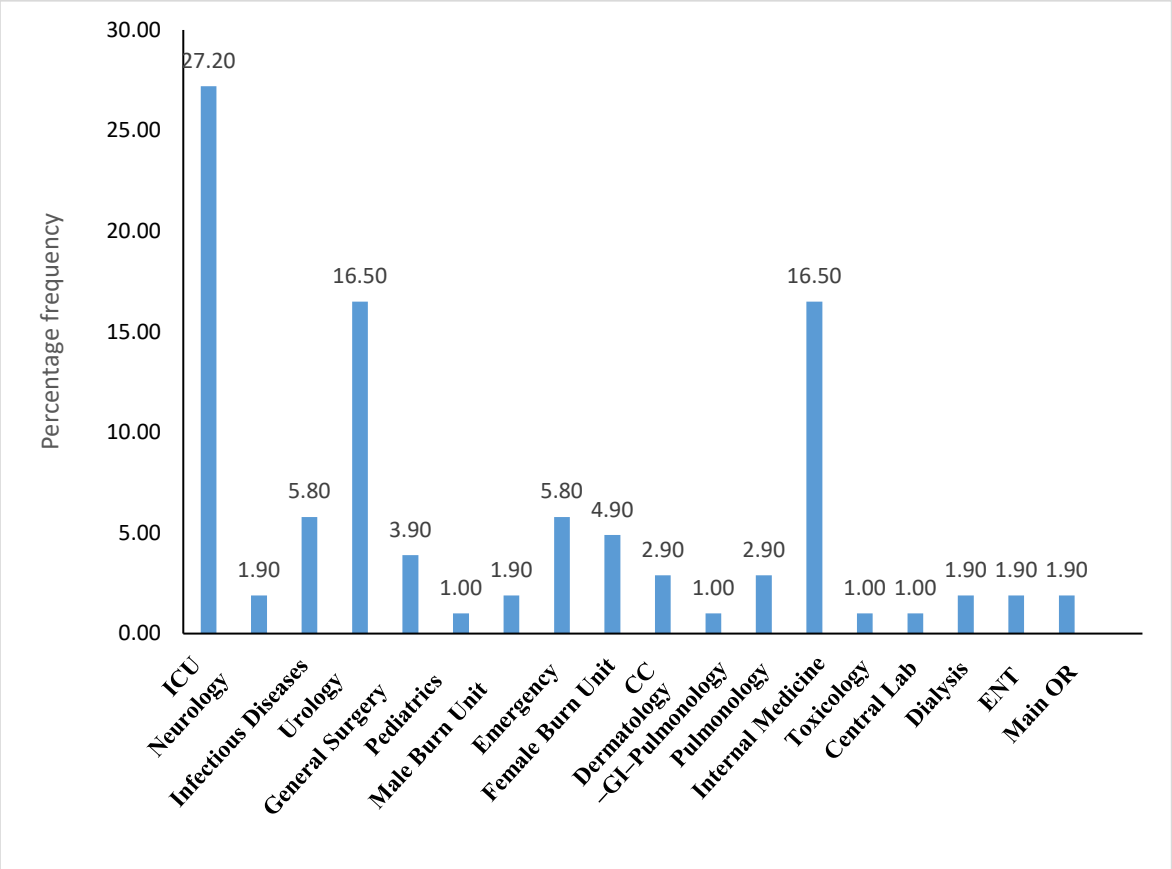

Figure 1. Distribution of *P. aeruginosa* clinical isolates across different hospital wards. Data are presented as percentages of the total number of isolates (n = 103).

Among the 103 clinical isolates, 67 (65%) were obtained from male patients and 36 (35%) from female patients. Gender distribution varied across hospital wards. Male predominance was observed in the urology and internal medicine wards (76.47% each), while female predominance was noted in the liver and gastroenterology ward (100%), the female burn unit (80%), and the CCU (66.7%). In the ICU, isolates were more frequently recovered from male patients (57.14%) than females (42.86%). Several awards, including infectious diseases, general surgery, and central operating rooms, showed an equal gender distribution (50:50).

Antimicrobial Resistance Profile

Antimicrobial susceptibility testing was performed to evaluate the resistance profile of the *P. aeruginosa* isolates. Among the β-lactam antibiotics, the highest resistance rate was observed for ceftriaxone (100%), followed by ceftiofur (93.2%). High resistance rates were also detected for ceftazidime (69%), cefepime (68.25%), piperacillin/tazobactam (65%), and imipenem (69%). Among the non-β-lactam antibiotics tested, resistance rates were 73% for ciprofloxacin and 62% for amikacin. Aztreonam showed the lowest resistance rate (54.4%) among the β-lactam agents evaluated. In terms of susceptibility, amikacin (35%), ceftazidime (29.37%), aztreonam (29%), imipenem (28.57%), and piperacillin/tazobactam (28.57%) demonstrated relatively higher activity against the isolates. Intermediate susceptibility was most frequently observed for aztreonam (16.5%), followed by piperacillin/tazobactam (6.35%), whereas other antibiotics showed minimal intermediate responses.

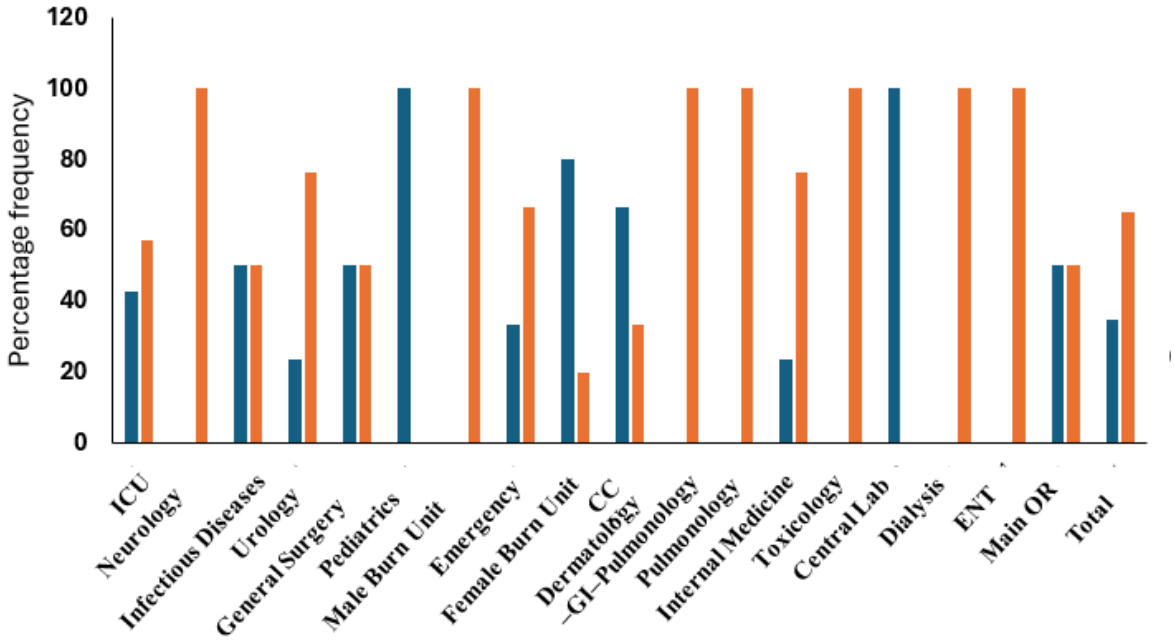

Figure 2. Percentage distribution of bacterial isolates obtained from different hospital wards according to gender.

Relative Expression of *bla*$_{SHV}$ Gene

The relative expression of the *bla*$_{SHV}$ gene was assessed in 20 selected *P. aeruginosa* isolates, including 17 imipenem-resistant and 3 imipenem-susceptible strains, using RT-qPCR. Gene expression levels were normalized to the *16S rRNA* housekeeping gene and calculated relative to the reference strain *P. aeruginosa* PAO1 using the $2^{-}\Delta\Delta Ct$ method.

All clinical isolates demonstrated significantly higher *bla*$_{SHV}$ expression compared with PAO1 ($p < 0.05$). Increased expression was observed in both imipenem-resistant and imipenem-susceptible isolates. Although expression levels varied among strains, the data indicate that *bla*$_{SHV}$ is transcriptionally active in the tested isolates, regardless of imipenem susceptibility status.

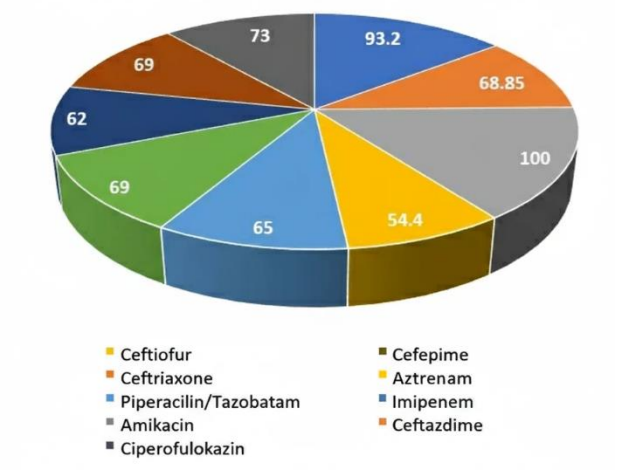

Figure 3. Percentage of antibiotic resistance among *P. aeruginosa* isolates to the antibiotics tested.

RT-qPCR Quality Control

Melting curve analysis demonstrated a single sharp peak for each primer set, confirming specific amplification of the target gene without primer-dimer formation or non-specific products.

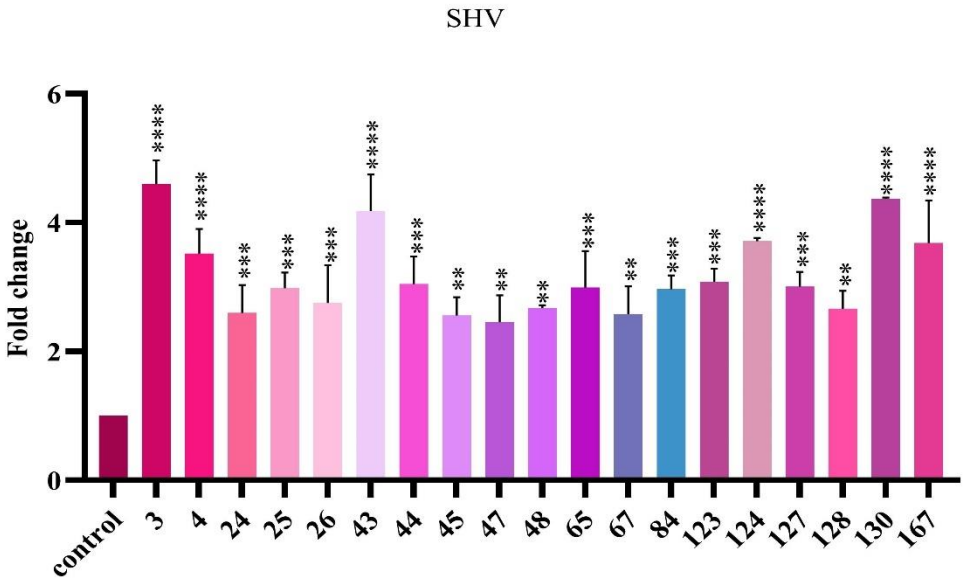

Figure 4. Distribution of *bla*$_{SHV}$ gene expression among imipenem-resistant isolates compared with three imipenem-susceptible isolates (Nos. 130, 124, and 26) and the reference strain.

Discussion

P.aeruginosa is a major opportunistic Gram-negative pathogen and a leading cause of healthcare-associated infections, particularly among critically ill and immunocompromised patients. Its remarkable capacity to acquire and express multiple antimicrobial resistance mechanisms has made this organism one of the most challenging pathogens in contemporary clinical practice (12). The present study provides an integrated analysis of hospital distribution, antimicrobial resistance patterns, and relative expression of the *bla*$_{SHV}$ gene in clinical *P. aeruginosa* isolates, with a particular focus on resistance to β-lactam antibiotics, especially cephalosporins.

The predominance of *P. aeruginosa* isolates in ICUs observed in this study is consistent with both national and international surveillance reports. ICU patients are frequently exposed to invasive medical devices, prolonged hospitalization, and repeated courses of broad-spectrum antibiotics, all of which generate strong selective pressure favoring resistant organisms. Previous studies have consistently identified ICUs, burn units, and high-dependency wards as major reservoirs for resistant *P. aeruginosa* strains (2,13). The high proportion of isolates recovered from ICU settings in the present study further reinforces the critical role of these environments in the persistence and dissemination of resistant strains.

Gender-based analysis revealed a higher overall prevalence of infection among male patients, a pattern that has been reported in several studies of nosocomial *P. aeruginosa* infections. This observation may reflect a combination of biological differences in immune responses, higher prevalence of underlying comorbidities among men, and increased exposure to healthcare settings. However, the reversal of this pattern in specific wards, such as female burn units, highlights the influence of ward-specific patient populations and clinical practices. These findings emphasize that local epidemiological dynamics must be considered when designing targeted infection control strategies rather than applying uniform policies across all hospital units.

The antimicrobial susceptibility profile identified in this study depicts a landscape concerning resistance. Complete resistance to ceftriaxone and very high resistance rates to other third- and fourth-generation cephalosporins indicate that resistance to these agents is highly prevalent among the studied *P. aeruginosa* isolates. Although intrinsic resistance to several cephalosporins is well recognized in *P. aeruginosa*, the magnitude of

resistance observed here suggests strong selective pressure driven by extensive and often empirical use of β-lactam antibiotics in hospital settings. Similar resistance trends have been reported in recent studies from Iran and other regions, indicating that this phenomenon reflects a broader regional and global pattern rather than a localized event (13).

For molecular analysis, a subset of isolates representing different imipenem susceptibility profiles was selected for RT-qPCR evaluation. Quantitative RT-qPCR analysis demonstrated a significant upregulation of the *bla*$_{SHV}$ gene in all tested clinical isolates compared with the reference strain *P. aeruginosa* PAO1. Increased expression of *bla*$_{SHV}$ suggests that enhanced production of SHV-type β-lactamases may contribute to the reduced susceptibility to β-lactam antibiotics observed among these isolates. SHV enzymes are known to hydrolyze penicillins and several cephalosporins, thereby compromising the effectiveness of commonly used β-lactam agents (14).

Notably, elevated *bla*$_{SHV}$ expression was also detected in isolates that remained phenotypically susceptible to imipenem. This finding is mechanistically plausible and clinically relevant. SHV-type enzymes generally lack efficient carbapenem-hydrolyzing activity; therefore, carbapenem resistance in *P. aeruginosa* typically requires additional mechanisms such as metallo-β-lactamase production, loss of outer membrane porins, or overexpression of efflux pumps (15). The presence of increased *bla*$_{SHV}$ expression in imipenem-susceptible isolates may therefore reflect selective pressure exerted by cephalosporins and other β-lactams without directly conferring carbapenem resistance.

Most previous studies from Iran and neighboring regions have primarily focused on the molecular detection of ESBL genes, including *bla*$_{SHV}$, without assessing their expression levels (16). The present study adds a quantitative perspective by demonstrating increased transcription of *bla*$_{SHV}$ in the analyzed isolates. This distinction is important because growing evidence indicates that the level of β-lactamase expression can influence the degree of antimicrobial resistance and may affect treatment outcomes (17).

Taking together, these findings highlight the high prevalence of cephalosporin resistance among the studied *P. aeruginosa* isolates and suggest that increased *bla*$_{SHV}$ expression may represent one contributing molecular mechanism. The results emphasize the importance of antimicrobial stewardship programs aimed at reducing unnecessary use of broad-spectrum β-lactam antibiotics, particularly in high-risk wards such as ICUs. In addition, molecular surveillance strategies that consider gene expression, in addition to gene detection, may provide a more detailed understanding of emerging resistance patterns.

This study has several limitations. First, *bla*$_{SHV}$ expression was evaluated at the transcriptional level, and enzyme activity was not directly measured. Second, other resistance mechanisms, including additional β-lactamase genes, efflux pump overexpression, and porin alterations, were not simultaneously assessed. Future studies integrating transcriptomic, proteomic, and phenotypic analyses may provide a more comprehensive understanding of β-lactam resistance in *P. aeruginosa*.

Conclusion

The present study highlights a concerning epidemiological and therapeutic profile of *P. aeruginosa* infections in the investigated hospital, characterized by a high prevalence in high-risk wards and extensive resistance to commonly used β-lactam antibiotics, particularly cephalosporins. Quantitative RT-qPCR analysis demonstrated significant upregulation of the *bla*$_{SHV}$ gene in the examined clinical isolates compared with the reference strain, suggesting that increased ESBL gene expression may contribute to the observed cephalosporin resistance. Notably, elevated *bla*$_{SHV}$ expression was also detected in imipenem-susceptible isolates, indicating that carbapenem susceptibility does not necessarily exclude the presence of β-lactamase–mediated resistance mechanisms. Overall, these findings underscore the value of integrating molecular analysis of resistance gene expression with routine antimicrobial susceptibility testing, alongside strengthened antimicrobial stewardship and targeted infection control strategies in high-risk hospital settings.

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