



Phenotypic Identification and Molecular Detection of *ecfX* and *lasB* gene in *Pseudomonas aeruginosa* from Burn Wounds with Molecular Redocking of Reference LasB Inhibitor

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Abstract

Pseudomonas aeruginosa is an important burn wound pathogen and the production of the secreted elastase LasB is a potential anti-virulence target. In this study, bacterial isolation, *ecfX* and *lasB* detection, and reference-ligand redocking against LasB, were performed. A single wound swab was taken from 310 burn patients, attending hospitals and private clinics in Wasit Governorate, Iraq from 12 January to 30 May 2026. The isolates were characterized by culture traits and VITEK 2 and later confirmed by conventional PCR. Using AutoDock Vina, the crystallographic inhibitor U7F was redocked with LasB (PDB: 8CC4). *P. aeruginosa* was isolated from 94 samples (30.3%; 95% CI: 25.5–35.7%). Isolation was higher in males than females (33.7% versus 25.9%), without a statistically significant association (OR: 1.45; 95% CI: 0.88–2.39; $P = 0.139$). All 94 of the isolates were also positive for both genes. A total of seven exported docking poses with a score range from -9.492 to -6.974 kcal/mol. Connectivity based RMSD showed the best crystallographic match with rank 4 ($0.55 \text{ \AA}$) while the best pose scored $6.64 \text{ \AA}$. These results suggest a broad distribution of *lasB* carriage in the collection of isolates and the presence of a docking score/geometric agreement mismatch. Prospective inhibitor screening requires further computational validation and detection of genes does not determine protease activity.

Keywords: *Pseudomonas aeruginosa*; burn wounds; *ecfX*; *lasB*; molecular docking; zinc metalloprotease

Introduction

Burn injuries compromise the protective skin barrier and create a susceptible environment for bacterial colonization and infection. Among the organisms associated with wound complications, *Pseudomonas aeruginosa* is particularly important because of its ability to persist in damaged tissue and produce extracellular factors that interfere with host defenses and tissue repair (Maslova et al., 2021). Experimental evidence demonstrates that its secreted proteases can disrupt processes essential for cutaneous wound healing, supporting investigation of the virulence determinants carried by burn-associated isolates (Țânțu

et al., 2026). The clinical importance of this pathogen is compounded by antimicrobial resistance, as reflected in the inclusion of resistant *P. aeruginosa* in the World Health Organization's 2024 bacterial priority pathogens list. These challenges support research that combines reliable bacterial identification with investigation of biologically relevant virulence targets (Organization, 2024).

Molecular detection provides a useful complement to phenotypic identification and enables targeted investigation of bacterial virulence genes. The *ecfX* gene, which encodes an extracytoplasmic function sigma factor, has been investigated as a species-discriminating marker for *P. aeruginosa* (Gicquel et al., 2013). Its diagnostic relevance is supported by studies demonstrating reliable amplification across tested *P. aeruginosa* strains and discrimination from other examined *Pseudomonas* species. Accordingly, incorporating *ecfX* into molecular characterization can strengthen species identification, while parallel assessment of a virulence-associated locus provides information about the pathogenic potential of the recovered isolates. Among these loci, *lasB* encodes LasB elastase, also known as pseudolysin, a secreted zinc-dependent metalloprotease implicated in host tissue damage (Lavenir et al., 2007). LasB contributes to pathogenicity through proteolytic effects on structural and immune-associated host proteins. Experimental studies have linked pseudolysin exposure to impaired cellular processes involved in wound repair, while more recent work has demonstrated degradation of cytokines and chemokines relevant to the inflammatory environment of non-healing wounds. Together, these findings provide a mechanistic rationale for investigating *lasB* in burn-associated *P. aeruginosa*. Nevertheless, detection of the gene establishes carriage of the targeted sequence and must be distinguished from evidence of gene expression, protein secretion or enzymatic activity (Prasad et al., 2020; van der Plas et al., 2025).

The contribution of LasB to host injury has also motivated the development of inhibitors directed against its proteolytic activity. Structure-guided research has identified LasB inhibitors and demonstrated preclinical activity, including improved antibiotic efficacy in an experimental lung infection model. Although these findings do not establish therapeutic effectiveness in burn wounds, they support LasB as a biologically plausible target for anti-virulence investigation. Within this framework, molecular docking can explore ligand accommodation within a reference binding site (Konstantinovic et al., 2023; Trott & Olson, 2010). Redocking a crystallographic inhibitor provides a preliminary assessment of whether a computational workflow can reproduce an experimentally observed binding geometry before it is applied to candidate compounds. Such an assessment should consider both structural agreement and pose ranking, rather than interpreting docking scores alone as evidence of inhibition (Eberhardt et al., 2021; Warren et al., 2006). This study aimed to determine the isolation frequency of *Pseudomonas aeruginosa* among burn patients in Wasit Governorate, Iraq; identify the isolates using phenotypic methods and conventional PCR targeting the species-associated *ecfX* gene; determine the prevalence of the *lasB* virulence gene among the confirmed isolates; and compare bacterial isolation proportions between male and female patients. Additionally, exploratory molecular redocking of a reference LasB inhibitor was performed to assess crystallographic pose reproduction and pose ranking as a preliminary evaluation of the computational workflow.

Materials and methods

Collection of samples

A total of 310 clinical samples were taken from patients of different ages and sex from burn sites for this study. Samples were swabbed and placed in sterile transport medium in sample containers, and sent to a research laboratory. The clinical samples were collected from January 12th to May 30th, 2026, from the hospitals and private clinics in Wasit Governorate.

Identification of *P. aeruginosa* Isolates

From the 310 clinical samples 94 *Pseudomonas aeruginosa* isolates were obtained. The clinical samples were directly cultured on MacConkey, blood and nutrient agar and incubated aerobically for overnight

and then re-cultured on cetrimide agar. These were then examined under the microscope and biochemically using VITEK 2 system for proper identification.

DNA extraction and conventional PCR

Examined isolates are used for DNA extraction with commercial purification system (Presto™ Mini gDNA Bacteria Kit, Geneaid Biotech Ltd, Taiwan) according to the manufacturer's instructions, which is designed to isolate Gram-positive and Gram-negative bacteria DNA. In addition, the samples were screened for genomic DNA on a 1.5 % agarose gel prior to their storage at -20°C for subsequent molecular studies.

Molecular diagnosis of *ecfX* and *lasB* gene

All bacterial isolates were tested for two genes: elastase gene (*lasB*), which is associated with virulence and *ecfX*, which is a diagnostic gene targeted to confirm the identification of *Pseudomonas aeruginosa*, by PCR. The lyophilized target genes were provided by Macrogen Company, Korea and there was a different pair of forward and reverse primers used for each target gene. The sequence of the designed primers are presented in Table 1.

Table 1. Primer sequences supplied by the investigator.

PCR Primer sequences supplied by the investigator:				
Gene	Primer	Sequence as supplied	Product Size	References
<i>ecfX</i>	Forward	CGTCTCGCATGCCTATCAGG	456 bp	This Study
	Reverse	CTGGCGATGTGCTTTTCCAC		
<i>lasB</i>	Forward	ACCTACAAGCAGGTCAACGG	524 bp	
	Reverse	AACAGGTAGAACGCACGGTT		

PCR Reaction Mixture and Amplification Conditions

Using a kit provided by TransGen Biotech (China), monoplex PCR was used to detect the virulence-associated gene *lasB* and the diagnostic marker gene *ecfX* with expected amplicon sizes of 524 bp and 456 bp, respectively. Reactions were performed in a 25 μL volume which included 12.5 μL of master mix, 1 μL of the respective forward primer (10 pmol), 1 μL of the respective reverse primer (10 pmol), 8.5 μL of deionized water and 2 μL of template DNA. DNA was extracted and the concentration was measured using a Quantus fluorometer prior to amplification. Thermal cycling parameters for each of the target genes is provided in Table 2. The PCR products were electrophoresed horizontally in 1.5% agarose gel, stained with ethidium bromide and exposed to UV light. The size of amplicons were estimated by comparing with a DNA ladder of 100-1500 bp.

Table (2): Programs of PCR thermocycling conditions

Monoplex Gene	Temperature (°C) / Time					Cycle No.
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	annealing	Extension		
<i>lasB gene</i>	95°C/3min	95°C/30sec	58°C/30sec	72°C/1min	72°C/3min	32
<i>ecfX gene</i>	95°C/5min	95°C/30sec	59°C/30sec	72°C/1min	72°C/5min	35

LasB structure preparation and reference-ligand docking

For molecular docking, the experimental LasB structure (PDB ID: 8CC4) that was determined at a resolution of 2.70 Å and corresponds to UniProt P14756 was selected in the present study (Konstantinovic et al., 2023; Manayia et al., 2025). The receptor was chain A, which contained 301 residues (298 modeled) in the deposited sequence of which the terminal residues were not resolved. The protein atoms, zinc and calcium ions from chain B and crystallographic inhibitor U7F and water molecules from chain A were

removed for receptor preparation, hydrogen was added and charges assigned with the server preparation workflow and no local energy minimization was done. The inhibitor U7F from the co-crystallized structure was chosen for the reference-ligand redocking. According to the RCSB chemical-component record (Zardecki et al., 2016) its molecular formula and reference molecular weight were C₁₃H₁₇F₃NO₄P and 339.247 Da, respectively. The SMILES to generate the ligands in a neutral reference state is: CC(C)C[C@H](C(=O)Nc1ccc(cc1)C(F)(F)F)P(=O)(O)O. Only the computational analysis was done for U7F, and it was not done for other candidate compounds.

Docking was carried out using docking software (AutoDock Vina 1.2.7) using Dockify platform with Vina scoring function (Eberhardt et al., 2021; Trott & Olson, 2009). The coordinates of the three dimensional ligand were generated with Open Babel with `-gen3d` option and then the pH was adjusted to 7.4 and the ligand was prepared for PDBQT conversion [12]. The pH level of the receptor was also set at 7.4. The receptor was treated as rigid, and the search box was centered at x = 10.504, y = 4.831, and z = 3.511 Å, with dimensions of 23.7 × 24.7 × 26.1 Å. The exhaustiveness was set to 2, the number of binding modes to be requested was set to 10, energy range to 3 kcal/mol, grid spacing to 0.375 Å and number of CPUs to 6. The seed value was set to be 0 and the execution log indicated that the seed generated was -1462140433. The Dockify job identifier was b5d437ac-310f-4876-9d83-ed7904ccfe3. The log of preparation contained an aromatic kekulization warning, and another cofactor conversion step. The processed receptor was unavailable (version of Open Babel) and the protonation state of the ligand also was not available. Therefore, the final incorporation and the atom type of the metal ions could not be determined and dedicated zinc-aware parameterisation was not reported (Santos-Martins et al., 2014).

Docking-pose assessment

The CSV, SDF and PDBQT exports had 7 poses whereas the execution log had 9 modes. Thus, only the 7 exported poses could be analyzed using a coordinate-based analysis. Vina RMSD lower and upper bounds were not used to assess the crystallographic ligand binding geometry, but rather as deviations from the highest ranked docked pose. For each of the 22 heavy atoms of the exported U7F pose, the element identity and undirected bond connectivity were compared to the heavy atoms of the crystallographic ligand attached to chain A, giving 144 possible correspondences between heavy atoms. The minimum RMS distance of these mappings was maintained for each pose, no rotation, translation or fitted superposition were needed. This was named connectivity-based RMSD (cRMSD) because the atom mapping did not require bond order, protonation state or stereochemistry to be equivalent. The cRMSD analysis was performed with the assumption that the exported poses were in the same coordinate frame as the original receptor; this could not be confirmed without the final processed receptor. The docking scores, pose rankings and cRMSD values were descriptively analyzed and the pose with the best cRMSD value was distinguished from the top-ranked pose.

Statistical analysis

The data analysis was done in python and the SciPy library (version 1.17.0). No attempts were made to complete missing data in all the aggregate statistics. Bacterial extraction ratios and 95% Wilson confidence intervals (NCIs) were calculated for each group using the number of patients in the group as denominator. 95% two-sided Klopfer-Pearson exact NCIs were used to determine the gene positivity of the 94 isolates extracted. Pearson's chi-squared test for independence, without continuity correction, was used to test the relationship between recorded sex and bacterial extraction, because the number of individuals per cell was > 5. A two sided Fisher's exact test was used as sensitivity analysis. The male/female odds ratios and extraction ratios are displayed with 95% log valued NCIs. Absolute differences in extraction ratios are reported as percentages and Newcomb confidence intervals were computed with Wilson limits. Proportionality ratios are not prospective risk ratios, but rather cross-sectional comparisons. All P-values were two-way, and effect estimates and confidence intervals were reported to determine the statistical significance of P ≤ 0.05. Alternative constructs that were generated by

the same computational search were considered docking positions, and scores were presented as a set. Observational reporting was done following STROBE guidelines if applicable.

Results

Phenotypic and Biochemical Characterization

The 94 *P. aeruginosa* strains were obtained from 310 clinical samples using morphological and culture characterization tests. The data obtained in this study revealed that *P. aeruginosa* infection rate in burn patients was high (30.32%) and 216 (69.677%) were other species of bacteria and non-grown. Additional studies are warranted to explore sources of infection and prevention. The results are similar to those reported by other authors who have indicated that burns are a high-risk area for the colonization of *P. aeruginosa* because burns are associated with impaired immune function and extended hospitalizations. All *Pseudomonas aeruginosa* were grown on MacConkey, blood, nutrient and cetrimide agars. In the microscopy, all 94 isolates were gram negative, rod shaped and red. All the isolates were able to grow at 42°C.

In this study, *Pseudomonas aeruginosa* was isolated from the burn samples which is easily reachable by it (30.32%). This was expected because it is so common in the community. This could be attributed to high population of immunocompromised patients in our community due to different ailments and environmental contamination in hospitals and the country, especially patients who are kept in the hospital for a long time. This may be attributed to overuse of antibiotics and differences in hospital infection control practices, local and environmental sanitation practices as well. This may also be attributed to the different sample sizes of these bacterial isolates in this study (Gomersall et al., 2023; Ozkurt et al., 2005; Quick et al., 2014).

In general, resistance to different antibiotics has been attributed to a variety of factors such as modifications of the target site, and cell membrane permeability (Tae et al., 2014).

Vitek-2 system was also used to identify the entire sample of suspected *Pseudomonas aeruginosa* and the culture result was positive to confirm the diagnosis. All the above tests confirmed that 94 isolates out of 310 clinical samples were of *Pseudomonas aeruginosa* and the Vitek-2 system tests confirmed the same.

Prevalence of *Pseudomonas aeruginosa* and Its *ecfX* and *lasB* Genes in Burn Patients

In the current study, a total of 94 *P. aeruginosa* were recovered from 310 clinical specimens from burn patients, with a prevalence rate of 30.3% (95% confidence interval [CI]: 25.5–35.7%). Other 216 specimens (69.7%) did not reveal any bacterial growth and/or other bacterial species were detected (Table 3 and Figure 1A). The results show that *P. aeruginosa* was found in about one-third of the samples tested and thus has been shown to be frequent among burn patients in the present study.

Table 3. *P. aeruginosa* recovery by recorded sex.

Group	Positive, n (%)	Negative, n (%)	Total	Positive 95% CI
Male	59 (33.7)	116 (66.3)	175	27.1–41.0%
Female	35 (25.9)	100 (74.1)	135	19.3–33.9%
Total	94 (30.3)	216 (69.7)	310	25.5–35.7%

In the present study, the prevalence of *P. aeruginosa* was numerically similar to the prevalence of burn samples in Duhok, Iraq (Ali & Assafi, 2024) by Ali and Assafi 27.0% (27/100). Mohammed Said (2026) found a higher prevalence of 37.5% (45/120) among patients with infected burn in Duhok (Said, 2026). The results show that there were no significant differences between the overall bacterial detection rates in all the Iraqi environment studied, but the number of these is not enough to conclude that there is a significant difference between studies. These differences could be attributed to differences in patient selection, burn characteristics, time of sampling, previous exposure to antimicrobial agents, laboratory

methodologies, but the effect of these differences on the differences cannot be evaluated from the combined data available.

The prevalence of *Pseudomonas aeruginosa* among male burn patients was 33.7% (59/175; 95% CI: 27.1–41.0%), compared with 25.9% among female patients (35/135; 95% CI: 19.3–33.9%), as shown in table 3 and figure 1B. The overall prevalence of positive cases was 62.8% in the male and 37.2% in females; the prevalence of each sex was calculated by the number of people examined in each sex.

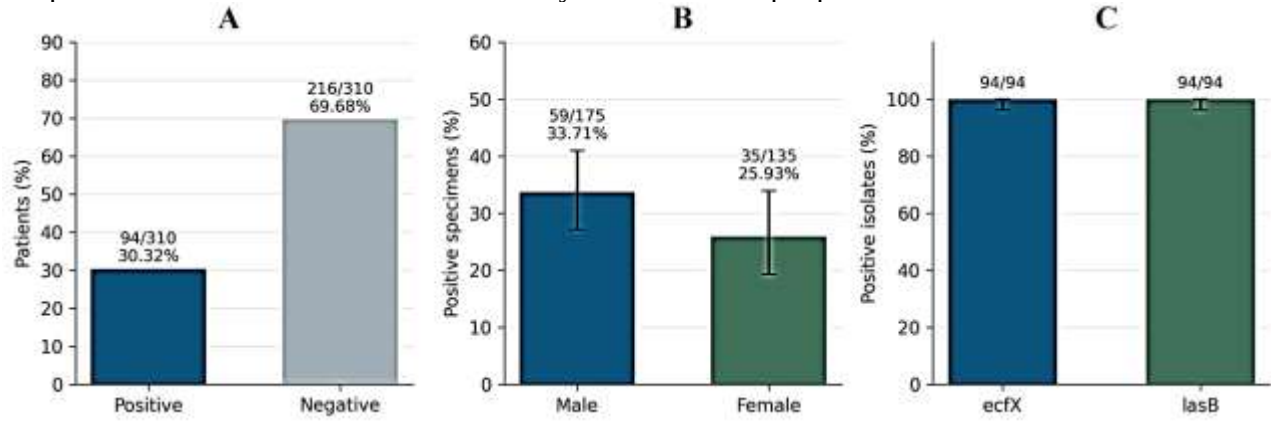


Figure 1. Prevalence of *P. aeruginosa* and detection of *ecfX* and *lasB*. (A) Overall prevalence among 310 burn patients; (B) sex-specific prevalence with Wilson 95% confidence intervals; (C) positivity for *ecfX* and *lasB* among 94 isolates with exact 95% confidence intervals.

Males were 1.45 times (95% CI: 0.88–2.39) more likely to have a positive test compared to females and had a positivity ratio of 1.30 (95% CI: 0.91–1.85) compared to females (Table 4). There was an absolute difference of 7.79 percentage points (95% CI: –2.56 to 17.66). These suggested that males had a higher prevalence, but that the null estimates were within the confidence intervals. Pearson's chi-square test did not show any statistically significant association between sex and detection of *P. aeruginosa* ($\chi^2 = 2.188$, $df = 1$, $P = 0.139$) confirmed by two sided Fisher's exact test ($P = 0.170$). Thus, the higher prevalence in males was only descriptive and not statistically significant evidence of an association with sex. However, the nonsignificant results do not suggest an equal prevalence in the sexes as the intervals are compatible with both directions.

Table 4. Comparison between in males versus females

Measure	Estimate	95% CI / test result
Odds ratio	1.45	0.88–2.39
Positivity ratio	1.30	0.91–1.85
Absolute difference	7.79 percentage points	–2.56 to 17.66
Pearson chi-square	2.188; $df = 1$	$P = 0.139$
Fisher exact test	Two-sided	$P = 0.170$

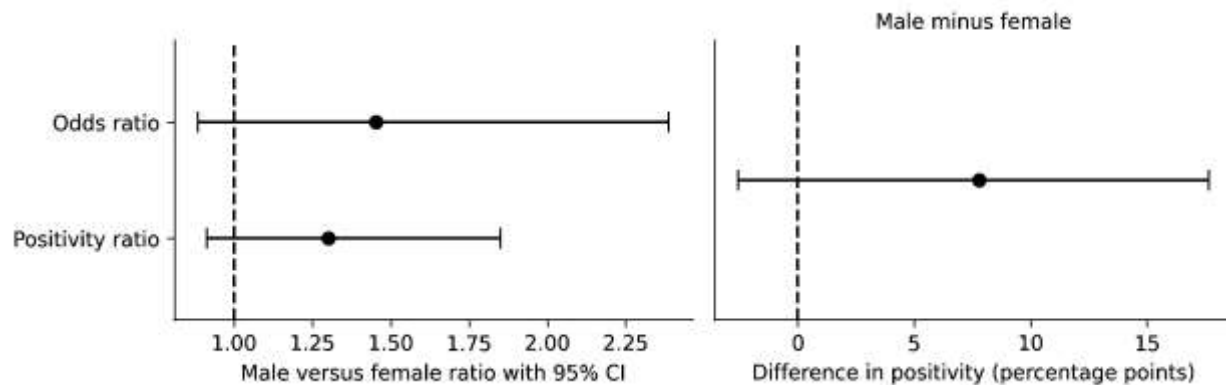


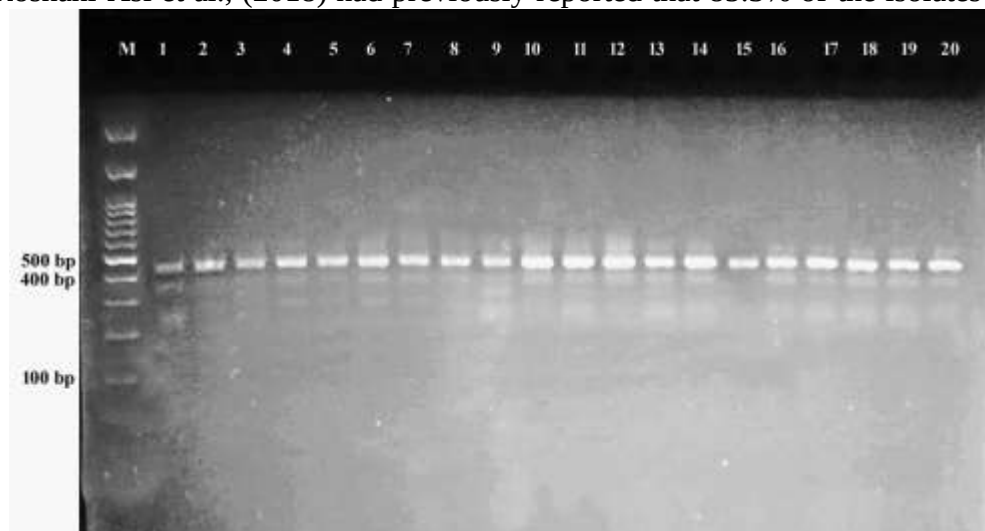
Figure 2. Estimates of the male versus female incidence effect. Relative estimates are shown with 95% confidence intervals and a zero value of 1; absolute difference is shown as percentage points with a zero value of 0. Intervals include their corresponding zero values.

In the present study, there was a higher prevalence among males than Mohammed Said (2026) who reported a significant higher prevalence of *P. aeruginosa* among females (45.7%) as compared to males (26.0%) (Said, 2026). These various patterns suggest that there may be a different correlation between sex and bacteria detection in the clinical populations. Not investigated in the present study, this might be attributed to varying exposures to the burn, severity, hospitalizations and history of treatment. Furthermore, statistically significant in one study and not in the other does not mean that it is statistically significant difference between the two studies.

Molecular detection

The presence of *ecfX* and *lasB* in all 94 *Pseudomonas aeruginosa* strains resulted in 100% detection for each gene (exact 95% CI: 96.2–100%) in the strain collection. The anticipated amplicon sizes were 456 bp for *ecfX* and 524 bp for *lasB* and representative electrophoretic profiles are shown in Figure 3 and Figure 4. All the isolates showed the presence of *ecfX*, therefore confirming their phenotypic identification as was done by (Lavenir et al., 2007) where they demonstrated that for the *P. aeruginosa* strains analyzed, an *ecfX*-targeted assay was reliable for their identification.

All the strains contained the *lasB* virulence associated gene indicating that this gene was universally carried in the strains of the present study. This was supported by the high prevalence of *lasB* (95.5%) seen by Mohammed Said (2026) which identified 21 isolates of *lasB* from 45 isolates identified and molecularly analysed. Roshani-Asl et al., (2018) had previously reported that 85.3% of the isolates of *P. aeruginosa*



from burn patients were positive for the presence of *lasB* (Roshani-Asl et al., 2018). These results show that the *lasB* gene is highly distributed among the burn isolates with different percentages of the burn isolates carrying the gene being found in the populations studied.

Figure (3): Agarose gel electrophoresis of PCR amplification products from patients group of *P. aeruginosa*, *ecfX* gene (1.5% agarose, TBE buffer (1X), 5V/Cm, 2hr.). L: The DNA molecular weight marker (100 bp ladder); lanes (1-20) positive amplification of ≈ 456 bp.

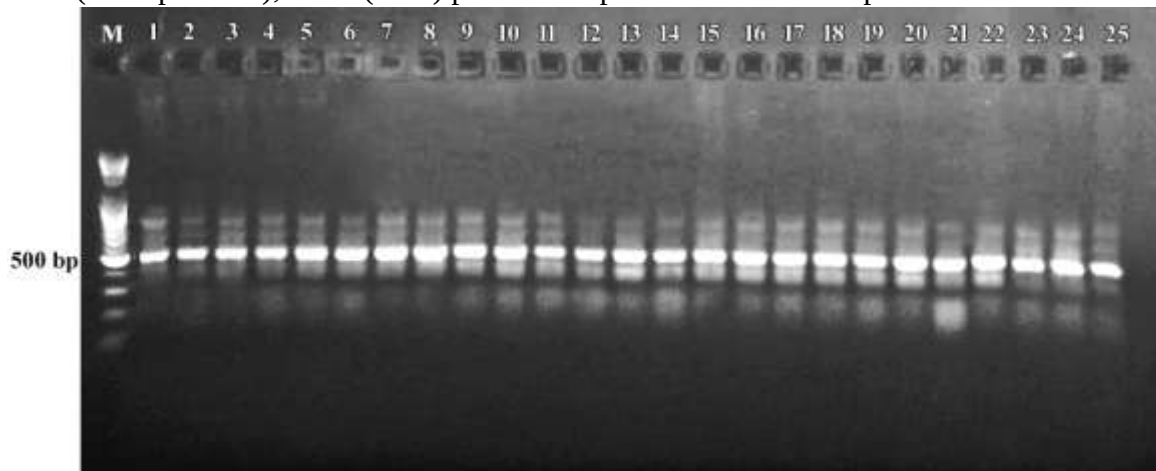


Figure (4): Agarose gel electrophoresis of PCR amplification products from patients group of *P. aeruginosa*, *lasB* gene (1.5% agarose, TBE buffer (1X), 5V/Cm, 2hr.). L: The DNA molecular weight marker (100 bp ladder); lanes (1-20) positive amplification of ≈ 456 bp.

Molecular Docking of U7F with LasB and Crystallographic Pose Agreement

Seven poses were exported after redocking U7F against LasB, and the scores of the poses ranged from -9.492 to -6.974 kcal/mol, with a median score of -8.378 kcal/mol. The cRMSD (root-mean-square deviation based on connectivity) from the crystallographic ligand varied between 0.55 and 7.92 Å with the median being 6.91 Å. The results of the individual docking scores and structural measurements are shown in table 5. The highest ranked pose had the best score of -9.492 kcal/mol and the cRMSD of 6.64 Å. In contrast, rank 4 was the most similar to the crystallographic ligand (0.55 Å), even though the score was less good (-8.378 kcal/mol). The difference in their docking scores was 1.114 kcal/mol. These results prove that the search sampled a near-crystallographic pose, but it wasn't the top hit.

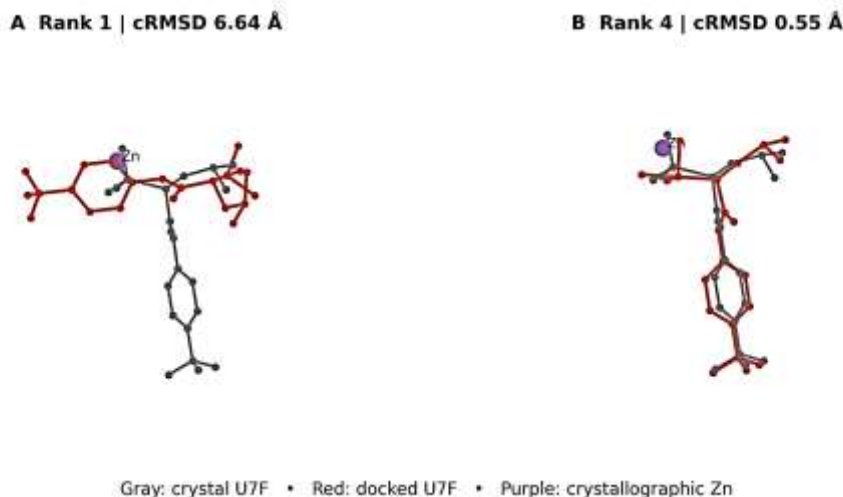
The structural overlays show the different rank orientations (1 and 4) with respect to the crystallographic ligand (Figure 5). The correlation between the pose ranking and the structural deviation is summarized in Fig. 6, and the protein–ligand structure is summarized in Fig. 7. A comparison of all seven exported poses is shown in Supplementary Figure 8.

Table 5. Docking scores and structural deviations of U7F poses against LasB.

Rank	Vina score (kcal/mol)	Vina RMSD lb / ub (Å)	cRMSD to crystal (Å)
1	-9.492	0.000 / 0.000	6.64
2	-8.924	5.273 / 7.897	6.91
3	-8.725	2.035 / 3.026	7.92
4	-8.378	5.188 / 6.762	0.55
5	-8.104	5.276 / 7.520	6.65
6	-7.745	5.915 / 7.778	7.34
7	-6.974	5.711 / 7.668	7.22

Vina RMSD bounds use the top-ranked docked pose as the reference; cRMSD uses the crystallographic ligand and permissive connectivity-based atom matching. Values describe alternative poses from one docking run.

Figure (5): Structural comparison of docked and crystallographic U7F. Overlays of the crystallographic



ligand (gray) with docked poses (red): (A) rank 1 and (B) rank 4. The crystallographic zinc ion is shown in purple. Coordinates are displayed without fitted superposition.

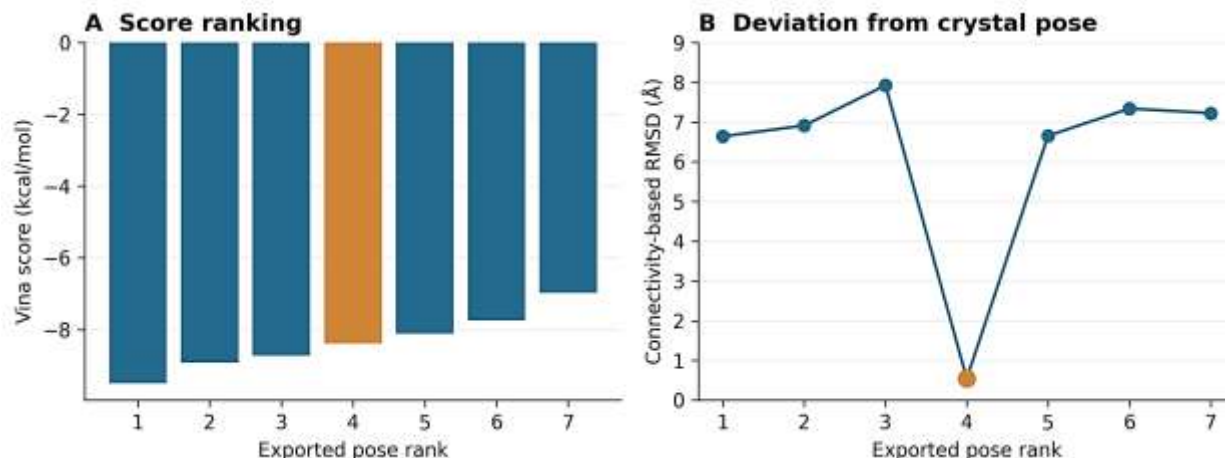


Figure (6): Docking scores and crystallographic deviations across U7F poses. (A) AutoDock Vina scores and (B) connectivity-based RMSD relative to the crystallographic ligand for the seven exported poses.

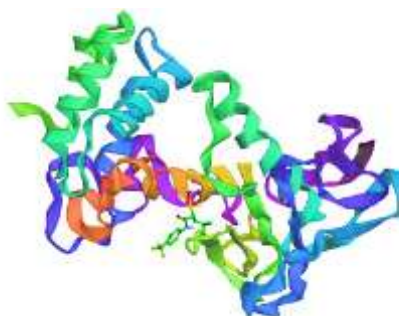
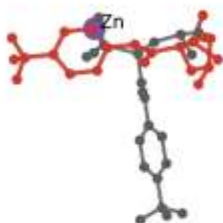
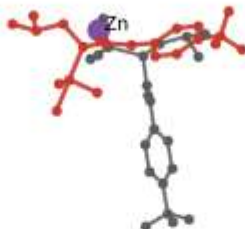


Figure (7): Structural overview of the LasB–U7F complex visualized in Dockify. The protein is represented as a multicolored ribbon and the ligand as green sticks. The displayed pose rank was not specified.

Rank 1 | -9.492 kcal/mol
cRMSD 6.64 Å



Rank 2 | -8.924 kcal/mol
cRMSD 6.91 Å



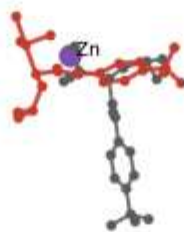
Rank 3 | -8.725 kcal/mol
cRMSD 7.92 Å



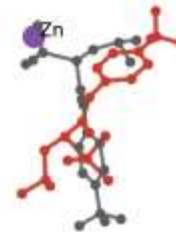
Rank 4 | -8.378 kcal/mol
cRMSD 0.55 Å



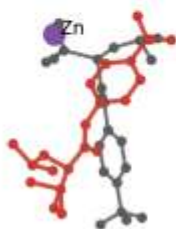
Rank 5 | -8.104 kcal/mol
cRMSD 6.65 Å



Rank 6 | -7.745 kcal/mol
cRMSD 7.34 Å



Rank 7 | -6.974 kcal/mol
cRMSD 7.22 Å



Crystal U7F: gray
Docked U7F: red
Crystal Zn: purple

Same receptor frame
No ligand realignment

Figure (8): Structural overlays of the seven exported U7F poses. Crystallographic U7F is shown in gray, docked poses in red and the crystallographic zinc ion in purple.

Discussion

In the present study, the isolation of *Pseudomonas aeruginosa* from burn wound samples was performed as well as the molecular detection of *ecfX* and *lasB*, and an exploratory redocking of a reference LasB inhibitor. The principle results were that the organism was isolated from approximately 1/3 of the patients sampled, the two genes were found in 100% of the organisms isolated and the docking score ranking was not consistent with the crystallographic ligand. These results are from the bacterial collection that was

investigated and provide a preliminary evaluation of the computational workflow that was used to investigate LasB.

The isolation rate (30.3%) was numerically very similar to that of Ali and Assafi (2024) who obtained 27.0% isolation rate for 100 burn samples collected from Duhok, Iraq (Ali & Assafi, 2024). Another recent study by Mohammed Said (2026) found that 37.5% of 120 patients with infected burns in Duhok, were infected with MRSA (Said, 2026). All the above observations were within the range of the above mentioned Iraqi clinical settings. However, there is no formal "between-study" comparison and numerical similarity is not necessarily statistically equivalent. Discrepancies between studies may be related to differences in the recruitment, burn severity, sampling time, and previous antimicrobial use and laboratory methods. So, the current estimate is a reflection of isolation in the sampled population and not of prevalence of invasive *P. aeruginosa* infection in Wasit Governorate.

Presence of *P. aeruginosa* in the samples of a burn wound is biologically relevant since damaged tissue is a location where bacterial factors may interfere with the wound repair process. Current observations, however, do not confirm the source of acquisition, nor the presence of contamination of the hospital environment or the use (or misuse) of antimicrobial agents or immunosuppression as a cause of the observed percentage of isolation. These explanations would be contingent upon the environmental sampling, treatment history or appropriate clinical measurements. Furthermore, other bacterial growth or no growth was found in the 216 samples that were negative for *P. aeruginosa* and these should not be categorized into microbiologically sterile. These data need to be understood in the light of isolation versus clinically diagnosed infections, specific to the organism.

There were more cases in males than in females but the statistical analysis did not show any association with sex. The 95% confidence intervals for the OR and PR included 1 and the interval for the absolute difference included 0. This is supported by the fact that Pearson's chi-square test and Fisher's exact test are in agreement. This finding is contrary to Mohammed Said (2026) who found females to be significantly more isolated than males (Said, 2026). This difference could be due to a difference in the clinical composition of the sampled populations and not to an intrinsic sex-dependent susceptibility. The predominance of males was not attributable to a biological cause as extent of burn, exposure and previous treatment were not available to adjust for. The nonsignificant association is not an indication of the equality of the isolation proportions of the two sexes.

All the isolates were detected by molecular method (ecfX) which corroborates with the culture based and Vitek 2 identification results. This was in line with the results of Lavenir et al. (2007) which have shown the potential of an ecfX targeting assay for the discrimination of the *P. aeruginosa* strains tested from other *Pseudomonas* species examined (Lavenir et al., 2007). It is not possible however, to automatically transfer the performance of a published assay to a new designed primer pair. The current 100% positivity is not an independent determination of diagnostic sensitivity/specificity as culture-negative samples and an appropriate non-target panel were not tested by the same molecular assay.

All the isolates studied contained the virulence associated locus as evidenced by universal detection of lasB. This is in line with the high level of detection of Mohammed Said (2026) who detected lasB in 21/22 isolates from which DNA was extracted (Said, 2026). But Roshani-Asl et al., (2018), have reported 85.3% isolates of *P. aeruginosa* associated with burns (QASIM et al., 2020; Roshani-Asl et al., 2018). In the comparison, it's stated that lasB is "frequently present in such collections, but not always. The proportions may vary due to variation in the strains sampled, selection of strains for molecular tests and characteristics of the tests. At present there is no data to distinguish these explanations.

The biological relevance of carriage of lasB is evidenced experimental data, that the protease coded by this gene is associated with tissue damage. In an experimental wound model, Prasad et al. (2020) demonstrated that exposure to pseudolysin and protease IV led to a decrease in several repair mechanisms, such as accumulation of collagen, neovascularization and epithelialization (Prasad et al., 2020). The findings here confirm the role of extracellular proteases in the pathogenesis of wounds; however, there

are no data presented to support that this was also seen in the patients that were included in this study. In the current study, it was not the production of enzymes, but the presence of genes that was measured, and the effects of the proteolytic activity and wound-healing outcomes. Thus, the molecular results suggest a potential virulence factor, and not to what degree this factor aids clinical damage.

More mechanistic information was offered by van der Plas et al. (2025) who showed LasB ability to degrade a wide range of extracellular cytokines and chemokines (including IL-6, TNF- α and IL-8) but not to depress the production of intracellular cytokines in the systems studied (van der Plas et al., 2025). This separation suggests that LasB could have an impact on inflammatory signaling, not only through altering the production of host cell cytokines, but also through extracellular proteolysis. This activity could be a potential link between bacteria's ability to make protease and abnormal host responses. But this mechanism can be only proven in the present isolates by gene carriage. Because of the possibility of identical endpoint-PCR results in the presence of differences in transcription, secretion, enzyme maturation and activity, universal lasB positivity cannot be correlated with uniform virulence.

The second methodological question was whether or not the chosen workflow would be appropriate for recapitulation of the experimental binding geometry of U7F in a reference structure of LasB. The most similar exported pose was the number 4 pose and the best Vina-scored pose was very different from the crystallographic ligand. This is a separation between the sampling of a credible geometry and the right ranking. This is in line with the overall methodological findings of Warren et al., (2006) who have performed a comparative study on the performance of docking and scoring, and concluded that no single method was good for all targets (Warren et al., 2006). Therefore, care should be taken not to consider the difference between the top and fourth ranked compound (1.114 kcal/mol) as an experimental difference in affinity.

The geometric evidence is good, in the sense that there is a low cRMSD rank 4, but at the same time is limited as to the method of comparison. Match of atoms was performed based on identity and connectivity of elements only, but not on the complete equality of bond order, protonation state or stereochemistry. Thus, cRMSD is just an agreement measure of coordinates and is not a full chemical validation of the binding pose. The interpretation must also be that the original coordinate frame was maintained, which wouldn't have been possible without the end result processed receptor. The near crystallographic pose is not necessarily the sign of the whole docking process, so don't use it to this end.

This is especially so for metal treatment as LasB is a zinc dependent protease. Santos-Martins et al. (2014) showed that zinc-specific docking parameterization was better to reproduce the zinc-coordination geometry than the general force fields tested in their work (Santos-Martins et al., 2014; Zubaidi et al., 2025). Zinc and calcium were kept in the first receptor preparation in the present workflow but there was no confirmation of their incorporation and atom typing from the processed files provided. In addition to preparation uncertainties are the aromatic-kekulization warning and unverified ligand protonation state. These factors can have an impact on the electrostatic interaction and can be taken into account as ranking but the output does not indicate which of these factors caused the discrepancy. Therefore, the automated labelling of interactions is not necessarily regarded as being verified hydrogen bonds or metal-coordination interactions.

Also only one run was searched and the exhaustiveness was low. The exported poses showed a near-crystallographic geometry, but it is unknown if this result can be reproduced by using independent searches. The seven poses are not experimental observations but rather are different outputs of the same search, of which a descriptive summary is provided, and are not inferential significance testing. The fact that there are only seven exported structures against nine modes logged, further restricts the assessment based on the coordinates. These restrictions are not related to the reliability of the computational work flow nor are they intended to modify clinical isolation and PCR results.

Several Las B inhibitor studies have been published, although not as strong as that provided here, which offer a rationale for the choice of this protein. Inhibitors have not only been developed, but also in the

same study, Konstantinović et al. (2023) demonstrated that LasB inhibitor could improve the effectiveness of antibiotics, such as levofloxacin, in a mouse model of lung infection (Konstantinovic et al., 2023). They conclude that there is potential for approaches using LasB as adjunctive, under the conditions tested. But in the present study, it was aimed to re-dock a known crystallographic ligand and not a new compound, biochemical inhibition or therapeutic activities. The results of the calculations should thus only be used to give an indication of skill in reproducing a reference pose and not as an indicator of the effectiveness of treatment for burn patients.

The only biological relevance between the clinical and computational aspects is the lack of them for susceptibility to inhibition of the isolates. Note that the presence of a *lasB* fragment does not mean that the complete sequence/presence of the ligand-binding site is present in the 94 isolates. In addition, there was no variation in the binary gene detection in this series and association tests were not meaningful between the binary gene and patient characteristics. Most importantly, a set of bacteria associated with a burn is characterised and a clear assessment of the pros and cons of the reference-ligand redocking is provided. A functional protease measurement and chemically verified and reproducible docking workflow would be ideal to increase the correlation between gene carriage and the assessment of inhibitors.

Conclusions

Pseudomonas aeruginosa was isolated from 30.3% of the sampled burn patients, with no statistically significant association between isolation and sex. All identified isolates carried *ecfX* and *lasB*, supporting molecular identification and demonstrating widespread carriage of the investigated virulence-associated gene. Reference-ligand redocking generated a pose close to the crystallographic geometry but did not rank it first, indicating that geometric sampling and score-based selection were not concordant. These support further investigation of LasB activity and inhibitor interactions while remaining insufficient to establish isolate-specific susceptibility, therapeutic efficacy or a fully validated screening workflow.

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