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Molecular Identification of *lasB* gene in *Pseudomonas Aeruginosa* Isolated From Patients With Urinary Tract Infection

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Abstract

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen capable of infecting various body sites due to the presence of multiple virulence factors that significantly contribute to disease. This study aimed to assess the antibiotic susceptibility of *P. aeruginosa* isolates, detect the frequency of the lasB gene in clinical isolates, furthermore analyze the genetic sequence and the resulting mutations.

Materials and Methods: A total of 120 samples were collected from patients with urinary tract infections (UTIs) during the period from November 2021 to March 2022. The specimens identified using microbiological and biochemical tests, as well as the VITEK2 system, and confirmed by PCR using specific primers. Gene sequence analysis was conducted by aligning the sequences with those in the gene bank using the Blast alignment program. Antibiotic sensitivity testing of *Pseudomonas aeruginosa* isolates was performed using the Kirby-Bauer method.

Results: Out of the 120 samples, 18 were positive for *P. aeruginosa* isolates. The infection rate was higher in females (88.8%) compared to males (11.2%). The highest resistance rate was observed for Levofloxacin (38%), while Piperacillin/Tazobactam and Meropenem showed the lowest resistance rates (11% each). The lasB gene was detected in all 18 isolates, and gene sequence analysis confirmed their identity as *P. aeruginosa*, matching the reference sequence in the gene bank. The gene sequence analysis of the *lasB* gene revealed the presence of five mutations,

Conclusions: This study demonstrated that *Pseudomonas aeruginosa* possesses several virulence factors, including the *lasB* gene. Sequencing analysis of the *lasB* gene revealed the presence of multiple mutations.

Keyword : *P. aeruginosa*, *lasB* gene, UTIs, PCR

Introduction

Pseudomonas aeruginosa is a pathogenic bacterium associated with various acute opportunistic infections, and it is characterized by a high mortality rate (1). In healthy individuals, *P. aeruginosa* infections typically occur in immunocompromised individuals, especially those in healthcare settings, where it can cause widespread hospital-acquired infections. The initial phase of *P. aeruginosa* infection involves attachment facilitated by flagella, pili, and the exoenzyme S. This bacterium is cytotoxic and promotes tissue necrosis (2). Subsequently, inflammation is mediated by virulence factors, including Exotoxin A, a macrophage toxin that inhibits protein synthesis by forming complexes with Elongation factor 2 (EF2) Additionally, endotoxins produced by *P. aeruginosa* contribute to fever, colonization, and local tissue invasion. The bacterium injects exotoxins (EXO-T, EXO-Y, EXO-U) via secretory systems and enzymes that degrade the host's defenses. Furthermore, the presence of the capsule plays a crucial role in evading phagocytosis and antibiotic action (3). UTIs are a significant driver of antibiotic usage and the development of antibiotic resistance. Therefore, it is crucial to comprehend the pathogenesis of these conditions to ensure appropriate management, benefiting both individual patients and controlling the spread of multidrug-resistant (MDR) organisms (4).

Classification of urinary tract infections can be based on their complexity, distinguishing between uncomplicated and complex cases. Complex UTIs often arise from factors such as the presence of urinary catheters, which expose the host to a higher risk of infection (5) . Elastin is a crucial component of human blood vessels, responsible for their elasticity. Elastase enzyme plays a significant role in determining the virulence of *Pseudomonas aeruginosa* bacteria during the infection stage (6). LasB are zinc metalloproteases that require calcium ions for stability. LasB has been reported to degrade mucins and surfactant proteins which collectively function to promote bacterial clearance (7). The proteolytic activity of the lasB mutant supernatant also could be enhanced, albeit to a lesser extent. Interestingly,

Furthermore, various *P. aeruginosa* virulence factors are attributed to specific genes, including the *lasB* gene, which encodes the enzyme lasB elastase, this enzyme actively participates in proteolysis and necrosis processes (8). LasB elastase is known for its high toxicity and ability to invade and damage tissues (6). It is noteworthy that the regulation of the *lasB* gene occurs through transcription mediated by the Quorum-Sensing system (9).

Materials and methods

Samples Collection

A total of 120 urine samples were obtained from patients exhibiting clinical symptoms of UTI and referred to the medical laboratories of three prominent hospitals in Al-Nasiriya city, namely Al-Hussein Hospital Teaching,

Al-Haboby Hospital, and Bint-Al-Huda. The collection period was spanned from 2022, to 2023. Following the conclusive diagnosis, 65 isolates of *E.coli* and 18 isolates of *P.aeruginosa* were identified, with 14 other distinct species.

Bacterial Identification

Microscopic examination: Bacterial colonies were identified by shape, color, and size, as well as the type of hemolysis, using blood agar, MacConkey agar, and Cetrimide agar. A single colony was selected, stained with Gram stain, and examined under a light microscope at 100x magnification, using oil immersion (10).

Biochemical tests: catalase, oxidase tests and VITEK-2 system were done to diagnose *P. aeruginosa*: (11).

Antibiotic susceptibility testing: To determine the Multiple Antibiotic Resistance (MDR) index for each isolate, the number of antibiotics were divided to which the isolate exhibited resistance by the total number of antibiotics tested (12). A MDR index greater than 0.3 suggests extensive utilization of the particular antibiotic within the environment from which the isolate originated.

DNA extraction: Genomic DNA was extracted from isolates of *P. aeruginosa* using the Genomic DNA Mini Bacteria Kit according to manufacturer's instruction .

PCR amplification: The *lasB* gene was amplified using forward primer 5-GGAATGAACGAAGCGTTCTCCGAC-3 and reverse primer 5- TTGGCGTCGAC GAACACCTCG-3 to amplify 284bp. The PCR amplification was done in a total volume reaction of 25 µl, including 100 pmol of each primer, a master mix containing PCR buffer, Taq polymerase, MgCl2, and dNTPs, and 100ng of template DNA. The amplification process involved an initial denaturation step at 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 30 seconds. A final extension step was performed at 72 °C for 10 minutes. The PCR amplicons were analyzed using 2% agarose gel electrophoresis, and photographs were taken under UV transillumination.

Sequencing: The sequencing of *lasB* gene was performed at Macrogen company using their genetic analyzer.

Results

The antibiotic susceptibility test was conducted for 10 antibiotics, and the results of the current study revealed that all *Pseudomonas aeruginosa* isolates exhibited significant variation in resistance to the antibiotics used in this study. These isolates showed a resistance rate of 20% to Ceftazidime, 22% to Cefotaxime, 29% to Pipracillin, 27% to both Ciprofloxacin and Tobramycin, 28% to Gentamicin, 21% to both Amikacin and Meropenem, 99% to Ofloxacin, and 9% to Imipenem.

Multiple Antibiotics Resistance

The findings of the current study revealed varying rates of multiple antibiotic resistance among the Pseudomonas aeruginosa isolates. The highest rate of multiple resistance, at 55.5%, was observed for a combination of antibiotics (PIP/TOZ, MEM, AK, CFX, AUG). Following closely, a rate of 33.3% was found for multiple resistance to antibiotics (PIP/TOZ, MEM, CTX). Conversely, the lowest rate of multiple resistance, equal to 11.1%, was observed for antibiotics (AUG, LEV, AK) individually.

Furthermore, multiple resistance to antibiotics (CIP, CFX) was found to be 22.2%, while the Pseudomonas aeruginosa isolates exhibited a multiple resistance rate of 33.3% for antibiotics (AK, LEV,

CIP). Additionally, a 22.2% rate of multiple resistance was observed for antibiotics (LEV, CTX). Lastly, the P. aeruginosa isolates showed a 22.2% rate of multiple resistance for antibiotics (AK, LEV).

Table 1: Multiple Antibiotics Resistance

No. of isolation	Multiple resistance	Number of antibodies	Percentage
1	AUG	1	11.1%
2	PIP/TOZ, MEM, CTX	4	33.3%
3	CIP, CFX	2	22.2%
4	PIP/TOZ, MEM, AK, CFX, AUG	6	55.5%
5	CIP, CFX	2	22.2%
6	LEV	1	11.1%
7	LEV, NOR	2	22.2%
8	AK	1	11.1%
9	CIP, CFX	2	22,2%
10	-	0	0%
11	AUG, NOR	2	22.2%
12	AK,LEV, CIP	3	33.3%
13	AK, NOR	2	22.2%
14	LEV,CFX	2	22.2%
15	AK, CTX	2	22.2%
16	LEV, CTX	2	22.2%
17	-	0	0%
18	AK, LEV	2	22.2%

Molecular detection of *P.aeruginosa* virulence gene *lasB*

The bacterial genome was isolated from positive cultures of *P.aeruginosa* then electrophoresis on 1% of agarose gel. As shown in figure (1), a 284 bp of *lasB* gene was amplified using polymerase chain reaction. PCR product was resolved on 2% agarose gel electrophoresis and detected by UV light transilluminator.

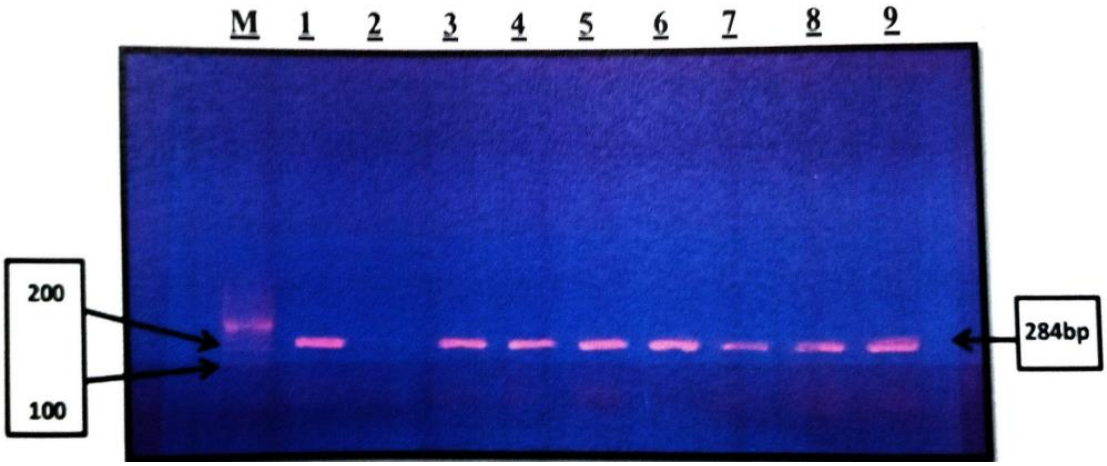
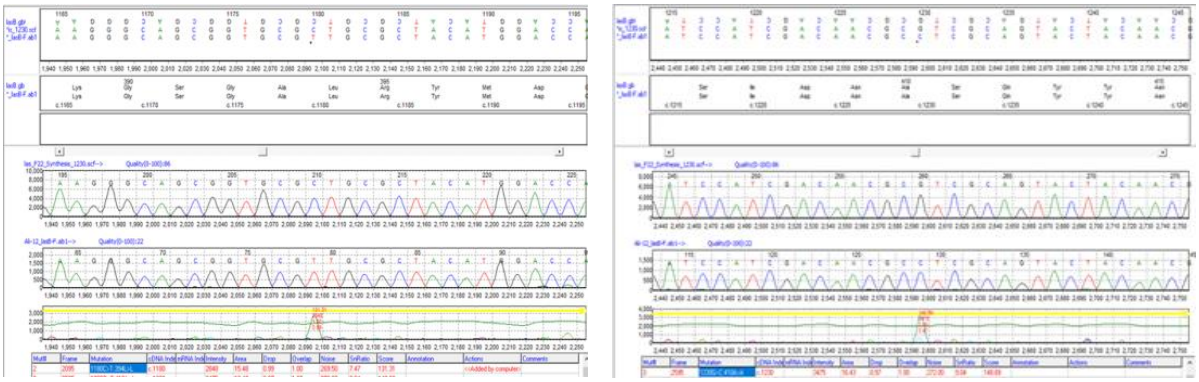


Figure (1) agarose gel electrophoresis of PCR product the *lasB* gene , M represents the ladder lane 2 represent negative result.

Sequence alignment of lasB gene

In this study eighteen isolates of *P. aeruginosa* , displaying an 88% similarity with the established reference sequence for *P. aeruginosa* archived in the gene bank through utilization of the Blast Alignment Program. Furthermore, the sequence scrutiny of the *lasB* gene divulged the existence of genetic mutations occurring at various positions. For instance, at position (1180 C>T) position 394 . Similarly, at position 1230, (1230 G>C) led to an analogous amino acid maintenance at position 410 (410 A>A). Moreover, position 1248 witnessed a (C>T) transformation, resulting in an unaffected amino acid status at 416 (416 G>G). Another alteration at position 1297 (1297 T>C), replacing Thymine with Cytosine, brought about a consistent amino acid composition at 433 (433 L>L). Likewise, a shift from Thymine to Cytosine at position 1305 (1305 T>C) culminated in an unaltered amino acid, denoted as Asparagine, at position 435 within the amino acid sequence.



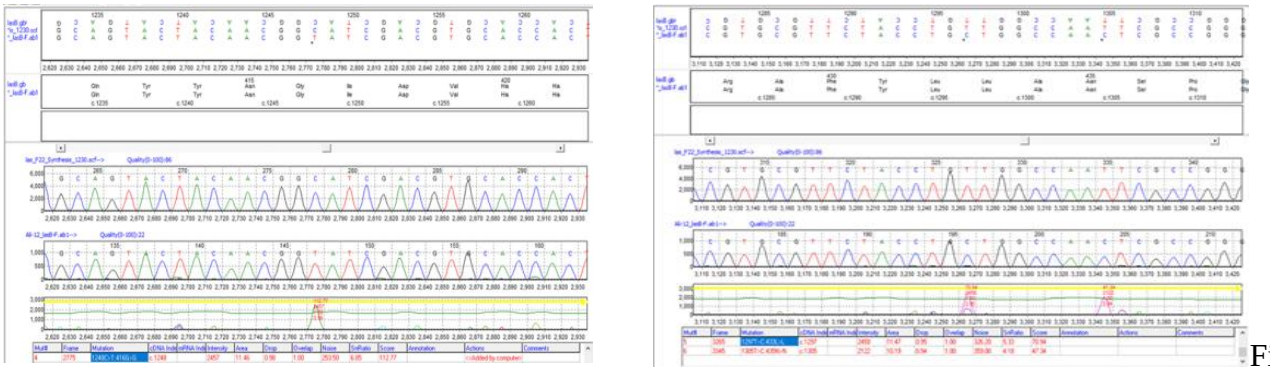


Fig 2: Alignment of *P. aeruginosa lasB* gene with reference sequence

Discussion

The outcomes of the biochemical assessments exhibited positive outcomes for the Oxidase and Catalase tests across all isolates. This positivity stemmed from the bacteria's capacity to generate the Oxidase and Catalase enzymes. Conversely, the IMVIC test suite, encompassing Indole production, Methyl Red (MR), Vocus-Proscar (VP), and Citrate utilization examinations, yielded predominantly negative results, except for the Citrate utilization test which yielded affirmative results. Notably, these findings concur with investigations conducted within Iraq (13**)**

The current investigation documented the percentage of resistance displayed by *P. aeruginosa* bacteria towards Fluoroquinolones, a class of antibiotics renowned for their efficacy against this bacterial strain. The results indicated the highest resistance of Levofloxacin (LEV) and Ciprofloxacin (CIP) (38% and 22%, respectively). Conversely, sensitivity rates for these antibiotics were 38% and 50%. Similar results were noted in a study by (14), which observed a 20% resistance rate to Ciprofloxacin among *P. aeruginosa* isolates. In contrast, a study by (15) indicated a resistance rate of 20.6% for the same antibiotic. Diverging from these findings, (16) reported significantly higher resistance (69%) to CIP among *P. aeruginosa* isolates. The mechanism underlying the resistance of these strains to Fluoroquinolones is attributed to mutations within the DNA gyrase enzyme, impeding DNA synthesis.

Conversely, the present study recorded the lowest antibiotic resistance rates for Piperacillin/Tazobactam (PIP/TPZ) and Meropenem (MEM), both standing at 11%. These findings align with (17), who documented an 11.3% resistance rate for MEM among *P. aeruginosa* isolates. In different results, (18) reported a remarkably higher resistance rate of 76% for MEM among Iranian isolates. Variances between these results appear substantial.

Resistance percentages for the antibiotics Cefotaxime (CTX) and Ceftriaxone (CRO) were 27% and 16%, respectively. These figures resonated closely with the outcomes of (15), who reported 100% resistance to both antibiotics. On the contrary, (19) encountered differing findings, with CTX and CRO resistance rates of 94% and 91%, respectively.

The findings unveiled that out of the examined samples, 18 belonged to the Pseudomonas aeruginosa bacterium, possessing the *lasB* gene at an 88% similarity rate. This gene encodes the production of the Elastase enzyme, as established by (6). closely resembling the outcomes of (20), who documented an 84.6% presence of the lasB gene among *P. aeruginosa* isolates. Elastase, a pivotal

virulence factor, facilitates the breakdown of Elastin protein within human blood vessels, responsible for their elasticity. As elucidated by (21), the Elastase enzyme significantly contributes to *P. aeruginosa's* virulence during the infection. The discrepancies in the detection of lasB, between different PCR-based studies, may be due to the techniques used or the primers employed. The data presented provide strong evidence that the lasB gene is present in the vast majority of *P. aeruginosa* strains (22).

Conclusions

The current study revealed varying rates of multiple antibiotic resistance among the *Pseudomonas aeruginosa* isolates. *lasB* gene one of the virulence factors which found in *P. aeruginosa* Sequencing analysis of the *lasB* gene revealed the presence of multiple mutations including transition and transversion.

Conflicts of Interest

There is no conflict of interests.

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