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Isolation and Molecular Identification of *Pseudomonas aeruginosa* from Clinical Samples Using PCR

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Abstract

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen responsible for diverse hospital- and community-acquired infections, particularly in immunocompromised patients and those with burn wounds, chronic illnesses, or implanted medical devices. Its multidrug resistance and virulence factors, including elastase (lasB) and exotoxin S (exoS), complicate treatment and increase morbidity and mortality. This study aimed to isolate and characterize clinical *P. aeruginosa* strains from various infections in Ramadi Teaching Hospital and to determine the prevalence of lasB and exoS genes using multiplex PCR.

A total of 305 diabetic foot ulcer (n=80) burns (n=90), and postsurgical isolates (n=305) were obtained. wounds (n = 75) and otitis infections (n = 60). *P. aeruginosa* confirmed use. biochemical assay. LasB was identified in 44.9% and exoS in 24.6% of isolates using Multiplex PCR. Both genes exoS and lasB were the most detected. In contrast, otitis isolates contained low exoS but a high prevalence of lasB detection with percentages 17.8% andv89.2% respectively. Both diabetic foot ulcers and post-operative wounds showed high lasB and moderate exoS. The kind of infection and elastase functions lead to difference in exoS expression. In this study PCR demonstrates its ability for rapid, sensitive detection of virulent genes, supporting targeted therapy and enhanced infection control. Epidemiological insights and clinical strategist targeting multidrug-resistant *P. aeruginosa* are based on mapping the distribution of lasB and exoS.

Keywords:
virulence genes, lasB, exoS, multiplex PCR, *Pseudomonas aeruginosa*, clinical infections.

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Introduction

The Gram-negative bacterium *Pseudomonas aeruginosa* grows in moist conditions. This opportunistic bacterium frequently causes infections in both the community and hospitals [1, 2, 26]. It often affects patient with weakened immune system, especially those with burn wounds, cystic fibrosis, implanted medical devices, or chronic illnesses. Bacterium causes a number of illnesses including urinary tract infections, pneumonia, sepsis, chronic lung disease, wound infections, surgical site infections, and ear infections [2, 3]. Treatment is always challenging because of resistance to many drugs, biofilm formation, efflux pumps, and production of degradative enzymes, highlighting the importance of rapid and precise detection to isolate cases and limit transmission [1, 3].

This pathogen can lead to several different infections, including urinary tract infections, pneumonia, bloodstream infections (sepsis), chronic respiratory conditions, surgical and wound infections, and otitis media. It occurs most in childhood and for adult it usually affects cystic fibrosis patients, contributing to higher morbidity and mortality [4]. Because of its inherent and acquired resistance to multiple antibiotics this led to severely limiting the treatments option. Biofilm formation that protects bacterial communities from antibiotics and immune defenses, the activity of efflux pumps that remove antimicrobial agents, and the production of enzymes such as beta-lactamases that degrade antibiotics all of this factor contributing to the resistance [2].

The rapid and accurate identification of *P. aeruginosa* in clinical samples is crucial due to its impact on public health and the elevated mortality associated with systemic infections. Timely diagnosis facilitates targeted antimicrobial therapy and enhances infection control, thereby preventing nosocomial outbreaks, a significant global issue.

Pseudomonas aeruginosa exhibits genetic unpredictability, thereby complicating its detection. This can influence that characteristic and the potential for other closely related species to mislead a diagnosis and result in incorrect treatment. Traditional identification in clinical samples primarily relies on phenotypic methods such as colony morphology, pigment production, motility assessment, and biochemical analyses [4]. However, the methods possess several limitations; they are time-intensive, frequently necessitating days to produce results, and may yield inaccurate outcomes due to strain-to-strain phenotypic variability or species similarity. Daily diagnoses have the potential to impact on patients, particularly those with compromised health or chronic conditions such as cystic fibrosis [5,6] and here there is an urgent requirement of quick, sensitive and reliable molecular means to detect and identify *P. aeruginosa* in clinical microbiology laboratories.

Polymerase chain reaction (PCR) is among the methods that are most commonly employed to obtain such requirements. PCR increases the number of genes that are unique to *P. aeruginosa*, through the application of specific DNA primers. PCR is able to detect faster and accurately in complex or mixed clinical samples. PCRs to differentiate *P. aeruginosa* and other species based on presence or absence of dene including outer membrane proteins (e.g. oprI and oprL) and virulence factors (e.g. toxA and other species-specific sequences such as ecfX and gyrB) using sensitivity and specificity [2].

Multiplex PCR amplifies a number of genetic targets simultaneously reducing the errors caused by genetic variation, gene transfer, or strain differences to improve diagnostic accuracy. Optimization of primer design and improved detection has been achieved through developed bioinformatics and whole-genome sequencing which enable results to be obtained in hours instead of the days, thereby speeding up the process of clinical decision-making and enabling immediate therapeutic intervention [7].

Multiplex PCR replicates the targets, which reduces the chances of false positive or negative due to genetic variations resulting in high diagnostic reliability. Regardless of these benefits, the use of molecular techniques in identifying *P. aeruginosa* in various clinical samples as a routine is still low in most health facilities.

This investigation using multiplex PCR to identify essential virulence genes in *P. aeruginosa* isolates of *P. aeruginosa*, with the view of offering a more precise diagnosis and prescribed therapeutic measures by incorporating both rapid molecular diagnosis and identification of virulence determinants, will ultimately benefit the patient outcomes, decrease unnecessary usage of antibiotics and help in the effective management of the infection process through improved infection control practices in a healthcare facility..

Objective

This paper aims to present a rapid and precise technique for identifying Pseudomonas *aeruginosa* in clinical specimens. Multiplex PCR mitigates the limitations of traditional methods by enabling the detection of essential virulent genes (lasB and exoS), thereby expediting diagnosis, informing suitable antimicrobial therapy, and improving infection prevention strategies. This data illustrates the significance of local data and the efficacy of molecular diagnostics in combating *P. aeruginosa* on a global scale.

Research Significant

The analysis of genes exoS and lasB and how in *Pseudomonas aeruginosa* from various clinical infections provides valuable insight into the disease-causing potential of each infection. Using sample obtained from different infections have more virulent genes can help in managing treatment, predicting how severe the disease might be, and supporting the treatment to be more specific. Multiplex PCR increases detection, supports traditional methods, and helps guide infection control for better patient care.

Literature Review

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen, common in hospitals, known for its antimicrobial resistance and ability to cause different type of infections [2]. Affected weak immune patient not only in humans but also in animals and plants, making treatment and control especially challenging [8].

Different methide available to detection of *Pseudomonas aeruginosa* such as molecular, immunological, and culture methods. Traditional culturing is standard, but it is slow, costly, and labor-intensive, especially for large numbers of ready-to-eat vegetable samples, emphasizing the need for faster molecular approaches like PCR [2]; [9]. *P. aeruginosa* is usually identified by its green pigment, but this method can be not accurate because if similar pigments from P. fluorescens can cause false positives, while some *P. aeruginosa* strains may not produce the pigment, leading to false negatives [10]. In samples with low bacterial levels or mixed infections the culture methods may fail to detect *P. aeruginosa* leading to delay diagnosis and treatment. This has increased reliance on faster, more sensitive, and specific molecular diagnostics [5].

The most advanced in the rapid and accurate detection of *P. aeruginosa* in clinical samples is PCR-based molecular techniques [7]. PCR methide skip the need of culturing by amplifying species-specific DNA, allowing accurate detection even in mixed samples or directly from clinical specimens [11]. The most common genes for PCR detection include ecfX, oprL, oprI, gyrB, and toxA. While oprL and ecfX showed notes for their high specificity and sensitivity, making them dependable markers in diagnostic assays [7]. Compared to traditional method, PCR assays targeting outer membrane protein genes like oprL offer higher sensitivity and specificity, effectively identifying *P. aeruginosa* in burn infections. Both Rapid PCR and qPCR allow real-time detection and quantification, supporting faster diagnosis and timely treatment [5].

Multiplex PCR allow to target multiple genes within one reaction, improves diagnostic accuracy by detecting both virulent genes and species-specific markers while reducing time and cost [12]. In *P. aeruginosa*, key virulence genes include lasB, producing elastase enzyme led to tissue damage, and exoS, which produces a toxin that disrupts host cells. Detecting these with multiplex PCR links gene presence to infection severity and outcomes, giving clearer insight into the pathogen's impact [13].

Multiplex PCR offers clear benefits; however, it faces challenges in primer design and optimization. Some limitation include primer competition, variable gene copy numbers, and sequence variations can lead to false or nonspecific results. The lack of a standardized protocol for *P. aeruginosa* detection led to limitation for clinical use [7]; [14]. Researcher still working to improve assay more reliably by testing primers on varied clinical strains and aiming checks for DNA quality and quantity [15].

Molecular detection of virulence genes provides insight into the distribution of pathogenic traits among clinical isolates [16]. within patient group, location, and infection type the prevalence of lasB and exoS varies. This variation helps assess risk and guide treatment, particularly for multidrug-resistant strains [17]. Another complementary molecular typing technique is Pulsed-Field Gel Electrophoresis (PFGE), which enables high-resolution genetic subtyping for outbreak investigations and population structure analysis [18]. PFGE is valuable for epidemiological typing of *P. aeruginosa*, however it is labor-intensive and unsuitable for rapid species or virulence detection. Combined with PCR, however, it provides a more complete picture for infection control [7]; [19].

In clinic using the molecular way to diagnose help to improve patient care by reducing the time from days to hours, directing targeted antibiotic medication earlier, and effectively putting infection control measures in place [20].

In summary, molecular methods, particularly multiplex PCR targeting species-specific and key virulence genes such as lasB and exoS, represent a rapid, precise, and informative approach for detecting *P. aeruginosa* in clinical samples. These methods overcome the limitations of traditional phenotypic assays by enabling earlier diagnosis, guiding appropriate therapeutic interventions, and improving infection control strategies. Addressing existing gaps in assay standardization and expanding local epidemiological data will enhance the clinical utility and global response to multidrug-resistant *P. aeruginosa* infections.

Material and Methodology

Study period and sample collection

This study was conducted from January to December 2020, during which clinical samples were collected from male and female patients admitted to hospitals in Ramadi, Iraq. A total of 305 *Pseudomonas aeruginosa* isolates were obtained from a variety of infection types, including otitis (n = 60), post-surgical wounds (n = 75), burns (n = 90), and diabetic foot ulcers (n = 80).

Isolation and Identification of *Pseudomonas aeruginosa*

Primary enrichment of clinical samples was performed by culturing on Luria Agar (LA) at 37 °C overnight. Presumptive colonies were subsequently subcultured onto chromogenic *Pseudomonas aeruginosa* selective agar to obtain pure isolates. Identification of *P. aeruginosa* was confirmed using a combination of morphological and biochemical assays, including triple sugar iron (TSI) agar test, indole production, catalase and oxidase activities, urease and hemolysin production, motility assessment, alkaline protease and lecithinase production, citrate utilization, nitrate reduction, oxidative-fermentative metabolism, and gelatinase liquefaction. These complementary tests ensured accurate phenotypic characterization of the isolates prior to molecular analysis.

DNA Extraction

A single purified colony was cultured in 10 mL of Luria Agar broth at 37 °C for 20 hours. Cells were harvested by centrifugation at 13,000 rpm for 3 minutes. Genomic DNA was extracted using a commercial DNA extraction kit (Promega, USA) according to the manufacturer's instructions. DNA quality and concentration were determined using a Nanodrop spectrophotometer and stored at –20 °C until PCR analysis.

Multiplex PCR Assay for Virulence Genes (lasB and exoS)

The PCR reaction was performed in a total volume of 25 µL, containing 12.5 µL GoTaq Green Master Mix (1st Base, Malaysia), 1 µL of each primer (100 µM) targeting the lasB and exoS virulence genes, 6.5 µL nuclease-free water, and 5 µL DNA template (10 ng). A negative control containing ultrapure sterile water was included in each run. Thermal cycling was carried out with an initial denaturation at

94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. PCR products were resolved on a 1.5% agarose gel in 1× TAE buffer at 100 V for 40 minutes, stained with MaestroSafe dye, and visualized using a Gel Documentation System (Syngene, USA). The expected amplicon sizes were 284 bp for lasB and 444 bp for exoS.

Statistical Analysis

All data were analyzed using IBM SPSS Statistics software (version 23). The Chi-square test and Risk Ratio (RR) were used to compare proportions. A p-value of < 0.05 was considered statistically significant.

Results

A total of 305 clinical isolates of *Pseudomonas aeruginosa* were obtained from various infections. These included 90 isolates from burn infections, 75 from post-surgical wounds, 60 from otitis infections, and 80 from diabetic foot ulcers.

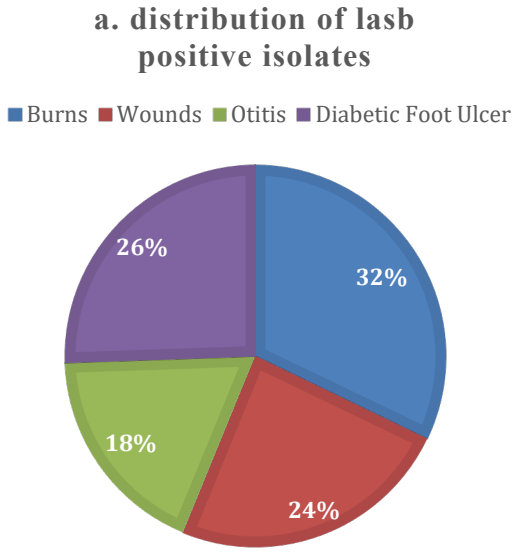
Biochemical Identification

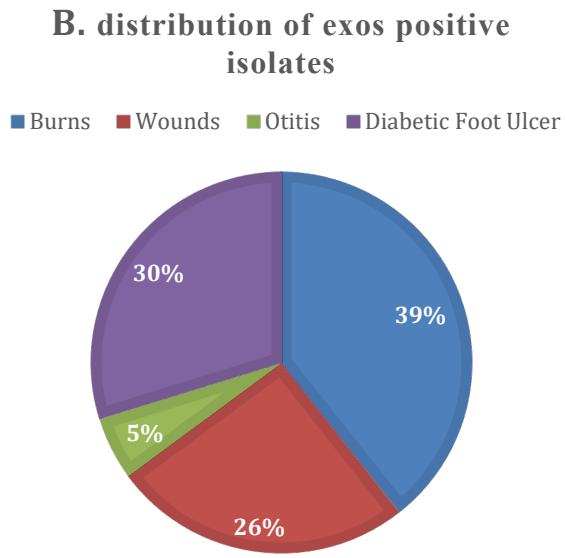
All 305 isolates were confirmed as *P. aeruginosa* through culture on chromogenic agar and standard biochemical tests. All isolates tested positive for catalase, triple sugar iron agar reactions, gelatinase liquefaction, oxidase activity, nitrate reduction, and motility. Conversely, the isolates were negative for indole, urease, alkaline protease, lecithinase, hemolysis, oxidative-fermentative activity, and DNase activity. These results validated the identity of the isolates before molecular analysis.

Virulence Gene Detection by Multiplex PCR

Multiplex PCR successfully amplified the target virulence genes, with lasB at 284 bp and exoS at 444 bp. The prevalence of these genes varied according to the infection source, as summarized in Table 1 below.

Figure 1. Distribution of lasB and exoS Virulence Genes in *P. aeruginosa* Isolates by Infection Source





Burn infections demonstrated the highest prevalence of virulence genes among all infection types. Otitis samples showed high lasB detection but very low exoS positivity. These results showed that lasB and exoS are main virulence factors in *P. aeruginosa* clinical isolates in Ramadi Teaching Hospital, with their prevalence varying depending on the infection source.

Discussion

In this study, clinical isolates of *Pseudomonas aeruginosa* of various types of infections in hospitals in Ramadi, Iraq, were isolated, identified and the molecular characteristics were characterized. The evaluation of virulence potential of *P. aeruginosa* strains associated with different clinical conditions was done by the combination of culture-based methods, biochemical assays, and a multiplex PCR that targeted two crucial virulence genes (lasB and exoS).

There were 305 *P. aeruginosa* samples sampled at various body locations such as burns, post-surgical wounds, otitis and diabetic foot ulcers. The high number of isolates of burn infections (90/305) is consistent with the fact that *P. aeruginosa* is a major opportunistic pathogen in the burn wounds [21]. Bacterial colonization and infection are idealized in burn wounds tissue due to necrosis as well as weakened immune systems [22]. In a similar manner, the sample of surgical wounds and diabetic foot ulcers reveals the ability of *P. aeruginosa* to take advantage of the impaired skin barriers and chronic wound environments which are commonly found in hospital settings [21].

All isolates were identified as *P. aeruginosa* by biochemical assays, which consistently produced positive results for motility, nitrate reduction, catalase, oxidase, triple sugar iron agar metabolism, and gelatinase liquefaction. These morphological traits

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are commonly used to differentiate *P. aeruginosa* from other Gram-negative bacteria that do not ferment [21]. The negative results for indole, urease, alkaline protease, lecithinase, hemolysin, oxidative-fermentative activity, and DNase further refine species differentiation and highlight the specificity of phenotypic tests when combined comprehensively.

In clinical, even with the advancements in molecular science the traditional method is still fundamental. The biochemical characteristics are in good agreement with the traditional characterization of *P. aeruginosa* [22]. The biochemical confirmation prior to PCR ensured high confidence in downstream molecular results.

A sizable subset of isolates had the lasB and exoS genes correctly identified by multiplex PCR. Overall, exoS was found in 24.6% of isolates, while LasB was found in 44.9%. in essential thing that contribute to *P. aeruginosa* pathogenicity are encoded by these virulence genes: exoS encodes an ADP-ribosyltransferase exotoxin released by a type III secretion system that interferes with host cell signaling and the cytoskeleton, while LasB encodes elastase, a protease that breaks down host tissues and immune effectors.

In this study multiplex PCR assay successfully detected the virulence genes lasB and exoS among the 305 *Pseudomonas aeruginosa* isolates from diverse clinical infections. The lasB was identified in 44.9% of isolates, while exoS was present in 24.6%. The lasB gene produces elastase, a zinc metalloprotease critical for degrading host structural proteins such as elastin and collagen, thereby lead to tissue invasion and immune evasion. In contrast, exoS encodes an ADP-ribosyltransferase exotoxin secreted through the type III secretion system that disrupts host cell signaling and cytoskeletal architecture [23]. When stratified by infection source, burn isolates exhibited the highest prevalence of both genes, with lasB detected in 95.6% and exoS in 80.4%. This high frequency highlights the aggressive virulence profile of *P. aeruginosa* strains causing burn wound infections, consistent with the known tendency of burn isolates to possess elevated levels of elastase and type III secretion effectors that promote extensive tissue damage [24].

Post-surgical wound and diabetic foot ulcer isolates similarly showed robust lasB expression (87% and 94.6% respectively) but somewhat lower exoS positivity (63% and 75.6%), suggesting that elastase-mediated proteolytic activity is a common virulence mechanism across wound types, while the deployment of exotoxins like exoS may be modulated by the specific host environment and immune status [13]. Remarkably, isolates from otitis showed a significant divergence, with a high lasB presence (89.2%) and a notably low exoS detection rate of just 17.8%. This pattern would indicate that the pathogen has adapted to the otic niche, where protease activity is more important for survival and colonization. However, exotoxin-mediated cytotoxicity is limited, perhaps as a result of immunological pressures inside the ear canal or distinct host tissue properties [24]. The varied distribution of virulence genes highlights the genetic diversity of *P.*

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aeruginosa and its ability to adapt its pathogenic traits to different clinical settings [23]. These results support the use of molecular diagnostics for infection management and epidemiological surveillance, as they are consistent with regional studies where virulence gene profiling has clarified pathogenic potential and illness severity [23, 24].

In summary, the high prevalence of lasB elastase and variable exoS positivity in *P. aeruginosa* isolates from Ramadi hospitals reflects critical virulence factors influencing clinical outcomes. These data validate the multiplex PCR approach for rapid detection of key pathogenic determinants, informing targeted therapeutic and infection control strategies.

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