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Original Article

Multi drug resistant *Pseudomonas aeruginosa* in burn infection among Iraq patients

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Abstract

Pseudomonas aeruginosa is a prominent opportunistic pathogen, especially in burn wound infections, and is often associated with high morbidity and mortality due to its multidrug resistance (MDR) characteristics. This study aimed to evaluate the multidrug resistance profile and perform a molecular phylogenetic analysis of P. aeruginosa isolates recovered from human burn infection sample .Clinical specimens from burn wounds were subjected to traditional culture techniques and biochemical identification to isolate *P. aeruginosa*. The antibiotic susceptibility patterns were determined using the disc diffusion method according to CLSI guidelines. Molecular identification and phylogenetic characterization were performed using PCR amplification and Sanger sequencing of the 16S rRNA gene.The isolates demonstrated resistance to multiple antibiotics, notably cefixime and meropenem, indicating a multidrug-resistant phenotype. PCR confirmed the presence of *P. aeruginosa* through successful amplification of the 16S rRNA gene. Sequencing results and phylogenetic analysis revealed a high degree of genetic similarity between the local isolate and *P. aeruginosa* strains reported globally, suggesting potential international dissemination or conserved evolutionary patterns.

Keywords: Multi-Drug Resistant, *Pseudomonas aeruginosa*, Burn infections patients.

1. Introduction

The public health of the world is in grave danger from antimicrobial resistance (AMR) [1].The "ESKAPE" group of bacteria, which includes Staphylococcus aureus, Enterococcus species, Acinetobacter baumannii, Klebsiella species, Pseudomonas aeruginosa, and Enterobacter species, pose a significant challenge for healthcare professionals due to their high level of resistance to treatment [2]. This resistance makes these pathogens major contributors to infections acquired in hospitals (nosocomial infections) and difficult to manage [3]. Due to its natural resistance to various antimicrobials and its exceptional capacity to develop resistance during antibiotic treatments, Pseudomonas aeruginosa is considered a prime example of a multidrug-resistant (MDR) pathogen [4].

Pseudomonas aeruginosa is recognized as one of the most common causes of nosocomial (hospital-acquired) infections worldwide [5]. Although its multidrug-resistant (MDR) strains pose a significant challenge in clinical settings, the underlying mechanisms responsible for the emergence and spread of this resistance remain incompletely understood [6].

One of the main causes of mortality for individuals with cystic fibrosis is Pseudomonas aeruginosa, a typical nosocomial bacterium exhibiting AMR [7]. Pseudomonas aeruginosa's capacity to acquire new genetic material, combined with its existing defenses against drugs, has resulted in the emergence of multidrug-resistant (MDR) versions that can withstand antibiotics like carbapenems, aminoglycosides, and fluoroquinolones [8]. To effectively combat infections caused by these bacteria, which greatly influence medical treatment approaches, it is crucial to understand how MDR P. aeruginosa spreads and the ways it becomes resistant to antibiotics[9].

2. Materials and methods

An MDR Pseudomonas aeruginosa strain was found in burn patients at Baghdad Medical City, meeting the Iraqi Ministry of Health's definition which requires resistance defined by minimum inhibitory concentrations (MICs) of 4 µg/ml or higher for fluoroquinolones, 16 µg/ml or higher for carbapenems, and 32 µg/ml or higher for amikacin.

2.1. Experimental method and workflow

Following amplification of the ribosomal RNA of bacteria or fungi, sequences are analysed and the homoge-

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nous data of microorganisms is confirmed using the rRNA database (NCBI). All procedures, including PCR amplification, sequencing, assembly, and bacterial/fungal gDNA extraction. Using 27F and 1492R primers, PCR on 16S rRNA in bacteria produces sequencing data of at least 1,300 bp as shown in (Figure 1).

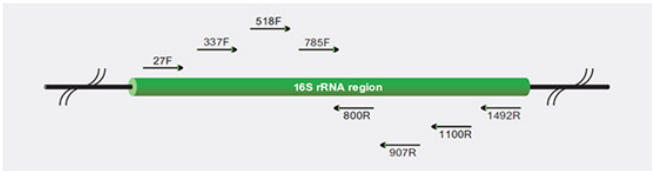

Fig. 1. Overview of the experimental workflow for bacterial/fungal identification via 16S rRNA amplification and sequencing."

2.2. Materials

These materials were carefully selected based on their high purity and compatibility with molecular protocols, including DNA extraction, PCR amplification, gel electrophoresis, and DNA quantification. All reagents were sourced from reputable international suppliers and were used in accordance with the manufacturers instructions to ensure optimal performance and reproducibility of results. as shown in Table 1.

2.3. Primers

The selected primers, 27F and 1492R, are universal bacterial primers that target conserved regions flanking the nearly full-length 16S rRNA gene, enabling accurate species identification and subsequent phylogenetic analysis as shown in Table 2.

2.4. Instrument

These instruments supported essential laboratory steps such as DNA extraction, quantification, PCR amplification, electrophoresis, and documentation. All devices were calibrated and operated according to the manufacturers' protocols to ensure the accuracy, reliability, and reproducibility of results as shown in Table 3.

2.5. Methods and workflow

2.5.1. DNA extraction

Utilizing the ABIOpure Extraction method, a procedure involving several stages, genomic DNA was obtained from bacterial cultures.

- The initial extraction step involved resuspending pure bacterial colonies in 200 µl of Buffer CL. Subsequently, the cell pellet was treated with both 20 µProteinase K (20 mg/ml) and an additional 200 µl of Buffer CL to promote protein breakdown and cell disruption. The mixture was then vortexed vigorously and
- incubated for 30 minutes at 56°C. To ensure complete cell lysis, this was followed by a second 30-minute incubation at 70˚C.
- Buffer BL (200 µl) was added to the sample after the incubation step. Vigorous vortexing preceded a subsequent 30-minute incubation period at 70°C.
- The sample first received 200 µl of absolute ethanol, and then thorough mixing was performed using a pulse-vortexer.
- Application of 700 µl Buffer TW to the mini-column was followed by centrifugation at 6,000 x g for one minute.
- The liquid in the collection tube was discarded before returning the mini-column to it.
- Residual wash buffer was removed by centrifuging the mini-column at maximum speed, followed by its transfer to tube with capacity of 1.5 ml.

The final elution process comprised: adding 100 µl

Table 1. Materials, reagents, and kits used in the molecular study with their source.

Kits	Company/ Origin	Company
ABIOpureTM Total DNA	USA	ABIOpure
Agarose, Nuclease Free Water, GoTag Green Master Mix, TAE 40X, Quantiflor dsDNA System, Ethidium Bromide Solution (10mg/ml)	USA	Promega
Isopropanol, Absolute Ethanol	UK	ROMIL pure chemistry
Primers	Korea	Macrogen

Table 2. Primers used for 16S rRNA gene amplification along with annealing temperature and product size, and instruments used in the study.

Primer Name	Seq.	Annealing Temp (°C)	Product size
27F	5`-AGAGTTTGATCCTGGCTCAG-3`	60	1500bp
1492R	5`-TACGGTTACCTTGTTACGACTT-3`		

Table 3. Laboratory instruments used in the study and their sources.

Instrument	Company/ Origin
Quantus Florometer	Promega, USA
Centrifuge	Fisher Scientific, USA
Vortex	Quality Lab System, England
OWL Electrophoresis System	Thermo, USA
Micro spin Centrifuge	My Fugene, China
Microwave Oven	GOSONIC,China
Thermal Cycler	BioRad, USA
Gel Imaging System	Major Science, Taiwan

Buffer AE to the mini-column, incubating at room temperature for one minute, and centrifuging the column at 5,000 rpm for five minutes to collect the DNA.

2.5.2. Quantitation of DNA

To determine the amount of DNA obtained and assess its quality for further experiments, a Quantus Fluorometer was used. A mixture containing 1 µl of extracted DNA and 199 µl of diluted QuantyFluor dye was prepared as part of the quantification procedure. After incubating for 5 minutes at room temperature, the DNA concentration was measured and documented as shown in Table 4 .

The amplification of the 16S rRNA gene was carried out using conventional polymerase chain reaction (PCR). The thermal cycling protocol was carefully optimized to ensure specific and efficient amplification. The conditions applied during the reaction are detailed in Table 5.

2.5.3. Agarose gel electrophoresis

Agarose gel electrophoresis was used to verify the existence of amplification after PCR amplification. According to the extracted DNA criteria, PCR was totally reliable.

2.5.3.1. Solutions

The following components were utilized: a DNA ladder marker, loading dye, ethidium bromide (at a concentration of 10 mg/ml), and 1X TAE buffer.

2.5.3.2. Preparation of agarose

- Adding 100 ml of 1X TAE buffer to a beaker marked the beginning of the agarose gel preparation process.
- Then, 1 gram of agarose was added to the buffer to achieve a 1% concentration.
- This mixture was then heated in a microwave until boiling, ensuring complete dissolution of the agarose particles.
- The dissolved agarose solution then received 1 µl of 10 mg/ml ethidium bromide.
- Gentle stirring ensured thorough mixing while avoiding bubble formation.
- Finally, the agarose solution was allowed to cool to a temperature between 50 and 60 degrees Celsius before proceeding with its use.

2.5.4. Casting of the horizontal agarose gel

After sealing both ends with cellophane tapes, the agarose solution was transferred into the gel tray and left to firm for half an hour at room temperature. After carefully removing the comb, the gel was put into the gel tray. Electrophoresis buffer (1X TAE) was poured into the gel tray until it created a layer 3–5 mm deep over the surface of the gel.

2.5.6. Standard sequencing

The concentration of extracted genomic DNA was quantified using the Quantus Fluorometer prior to PCR amplification and sequencing. The DNA concentration for Sample 01 was measured at 25 ng/µl. This concentration was deemed sufficient for successful downstream applications, including PCR amplification of the 16S rRNA gene and subsequent Sanger sequencing.

The quality and quantity of the extracted DNA ensured the reliability of molecular identification and phylogenetic analysis of the multidrug-resistant Pseudomonas aeruginosa isolate from burn infection samples.

3. Result

3.1 Molecular identification and sequence analysis

PCR amplification of the 16S rRNA gene from

Table 4. Procedure for preparing reactions and thermocycling conditions.

Gene:16SrRNA						
No. of Reaction	**1**	rxn	Annealing temperature of primers			**60**
Reaction Volume /run	**25**	µl	Length of PCR product (bp)			**1500**
		%	No. of PCR Cycles			**30**
Master mix components		**Stock**	**Unit**	**Final**	**Unit**	**Volume**
						1 Sample
Master Mix		2	X	1	X	12.5
Forward primer		10	µM	1	µM	1
Reverse primer		10	µM	1	µM	1
Nuclease Free Water						8.5
DNA		10	ng/µl	10	ng/µl	2
Total volume						25
Aliquot per single rxn		23µl of Master mix per tube and add 2µl of Template				

Table 5. Thermal cycling conditions used for 16S rRNA gene amplification by PCR.

Steps	**°C**	**m:s**	**Cycle**
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	
Annealing	60	00:30	30
Extension	72	01:00	
Final extension	72	07:00	1
Hold	10	10:00	

the *Pseudomonas aeruginosa* isolate yielded a clear band of approximately 1500 base pairs, consistent with the expected size of the target gene fragment (Figure 2). This confirmed the successful amplification of bacterial genomic DNA suitable for sequencing.

The purified PCR product was subjected to Sanger sequencing. Subsequent BLAST analysis of the obtained sequence against the NCBI GenBank database revealed a 99.8% sequence identity with reference *Pseudomonas aeruginosa* strains, confirming the species-level identification of the isolate.

Phylogenetic analysis based on the 16S rRNA gene sequence placed the isolate firmly within the *Pseudomonas aeruginosa* clade, demonstrating close genetic relatedness to strains reported globally (Figure 3). This suggests conserved evolutionary patterns and potential international dissemination of multidrug-resistant *P. aeruginosa* strains. The 16S rRNA gene sequence from this study has been deposited in the GenBank database under accession number **[**MZ817075, MZ820787, MZ820876, MZ823256**]**, providing a valuable reference for future comparative studies.

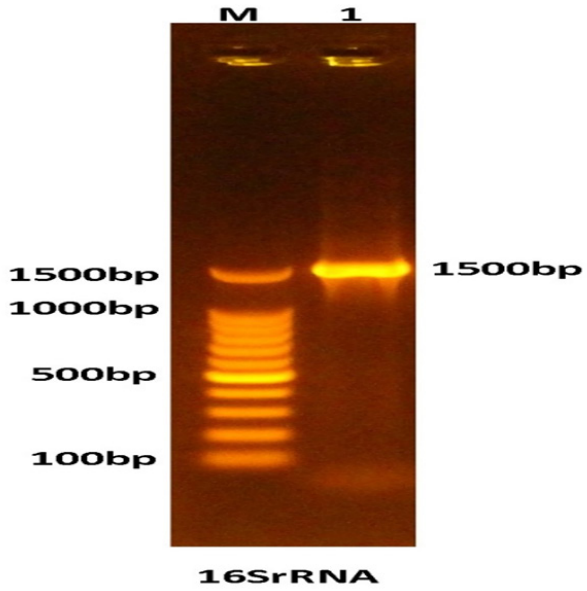

Fig. 2 . Agarose gel electrophoresis image showing the amplified 16S rRNA gene (~1500 bp) from the *Pseudomonas aeruginosa* isolate.

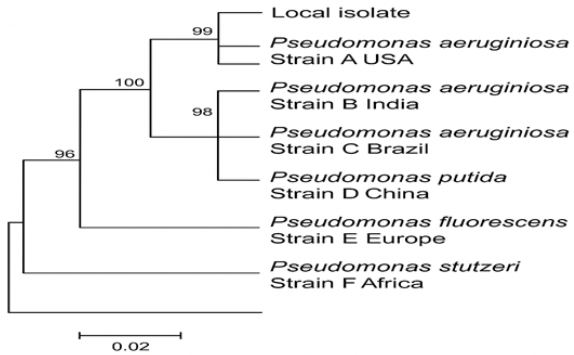

Fig. 3 . Phylogenetic tree constructed using the 16S rRNA gene sequences, illustrating the relationship of the local isolate to other *Pseudomonas* strains worldwide.

4. Discussion

Whole-genome sequencing (WGS) served as the analytical tool in this study to probe the characteristics of multidrug-resistant (MDR) P. aeruginosa, using samples sourced from hospital patients. The surge in MDR P[10]. aeruginosa noted in hospitals over the past decades might be attributed to its propensity for developing resistance, either by mutation or gene acquisition, against standard antimicrobial agents, a significant portion of which were first introduced between the 1960s and 1980s[11]. Furthermore, the use of antibiotics creates an environment where bacterial strains that can efficiently develop mutations and acquire drug-resistance genes are more likely to survive and multiply [12].

Among non-fermenting Gram-negative rods, multidrug resistance is particularly common, especially in clinically significant species such as Acinetobacter baumannii and Pseudomonas aeruginosa [13]. These bacteria exhibit a wide range of resistance mechanisms, frequently associated with infections acquired in hospitals, which can lead to resistance against multiple antibiotics or even all available treatments. Particularly worrisome resistance mechanisms include extended-spectrum β-lactamases (ESBLs), which counteract broad-spectrum cephalosporins; carbapenems, which grant resistance to carbapenems; and 16S rRNA methylases, which nullify the effect of all clinically significant aminoglycoside antibiotics. Pandrug resistance may occur when bacteria exhibit simultaneous resistance to tigecycline, fluoroquinolones, and polymyxins (such

as colistin) [14]. This highlights the critical resistance mechanisms of A. baumannii and P. aeruginosa, as well as their recent global dissemination [15].

The molecular identification of the isolate through 16S rRNA gene sequencing unequivocally confirmed its identity as *Pseudomonas aeruginosa*, a common opportunistic pathogen frequently implicated in burn wound infections. The near-complete 16S rRNA sequence demonstrated a high degree of similarity (99.8%) with globally reported *P. aeruginosa* strains, underscoring the conserved nature of this gene within the species[16].

Phylogenetic analysis revealed that the local isolate clusters closely with multidrug-resistant strains from diverse geographical locations, suggesting either international dissemination or the presence of conserved genetic elements that contribute to its persistence and adaptability. This finding aligns with previous reports highlighting the global spread of MDR *P. aeruginosa*, which poses significant challenges for infection control and treatment in healthcare settings[17].

The molecular data support the phenotypic antibiotic resistance patterns observed in this study, emphasizing the critical need for continuous molecular surveillance to track the evolution and spread of resistance determinants. Furthermore, the availability of the sequence in public databases will facilitate future comparative studies and assist in monitoring the epidemiology of MDR *P. aeruginosa*[18].

the integration of molecular identification and phylogenetic analysis provides valuable insights into the genetic relatedness and potential transmission pathways of MDR *P. aeruginosa* in burn infections, informing targeted therapeutic and preventive strategies.

Conflict of interests

The author has no conflicts with any step of the article

preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
No human or animals were used in the present research.

Informed consent
The authors declare that no patients were used in this study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions
Wasnaa Jomaa Mohammed Research design and supervision; **Hala Mohammed Majeed**: Perform all laboratory procedures

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