Original Article

Phylogenetic Origins of *Pseudomonas aeruginosa* Isolated from Various Clinical Samples in Iraq

Furqan Mohammed Al-Asady
Department of Pharmacy, Hilla University College, Babylon, Iraq

Abstract

Background: *Pseudomonas aeruginosa* is a significant pathogen associated with severe infections. **Objectives:** This study aims to investigate the phylogenetic origin of *P. aeruginosa* isolated in Iraq. **Materials and Methods:** A total of 150 clinical samples were collected from patients who visited hospitals in nine cities of Iraq between February 2022 and June 2022. Isolates were diagnosed using traditional methods and the polymerase chain reaction technique, and they were assigned designations from PA1 to PA46. Additionally, biofilm formation was assessed using microtiter plates, and the correlation between biofilm grade and antibiotic resistance was investigated. **Results:** Out of the 150 samples, 46 isolates (31%) of *P. aeruginosa* were obtained. Results indicated that 34 isolates (74%) were capable of producing biofilm. Fifteen isolates (PA1, PA2, PA3, PA5, PA6, PA7, PA8, PA9, PA11, PA12, PA16, PA19, PA23, PA24, and PA30), obtained from various regions of Iraq, were selected. Whole DNA extraction was performed on these isolates. Subsequently, sequencing of bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV} genes was conducted, and their genome locations were identified. Phylogenetic tree analysis was performed with the Molecular Evolutionary Genetics Analysis X 10.2.4 software program. The results revealed that one isolate (PA3) originated from the UAE, two isolates (PA6 and PA9) originated from India, three isolates (PA12, PA16, and PA19) originated from Egypt, and another three isolates (PA23, PA24, and PA30) originated from Iran. **Conclusions:** The study identified the presence of numerous isolates of *P. aeruginosa* collected from various regions of Iraq, with phylogenetic analysis indicating their origin from other countries.

Keywords: Clinical samples, Iraq, *P. aeruginosa*, phylogenetic origin

Introduction

Pseudomonas aeruginosa is a pathogen known to cause various diseases and is often resistant to many commonly used antibiotics.[1] It is responsible for infections in hospitals, including wound infections in immunocompromised patients.[2] Among Gram-negative opportunistic bacteria, *P. aeruginosa* is notably significant in nosocomial infections, commonly observed in burn and wound units.[3] Infections caused by this pathogen, particularly in burn patients with multi-drug resistance, pose significant challenges for treatment.[4,5] The presence of multi-drug resistant strains correlates with prolonged hospital stays and a considerable increase in inpatient mortality and morbidity.[6]

Antibiotic resistance is increasing significantly due to the widespread use of antibiotics like ciprofloxacin, β-lactamase, and aminoglycosides in burn units. This increase in antibiotic resistance is exacerbated by the limited availability and high cost of alternative treatments.[7] *P. aeruginosa* can develop a group of enzymes known as extended-spectrum β-lactamase (ESBLs), which can hydrolyze antimicrobial drugs, including penicillins, cephalosporins, monobactams, and carbapenems, thereby causing resistance to them.[8]

The most common ESBL genes found in *P. aeruginosa* are sulfhydryl variable (SHV), *CTX-M*, and *TEM* kinds, all of which are members of the SHV family. These enzymes trace their evolution back to the first plasmid-mediated β-lactamase discovered back in

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Address for correspondence: Dr. Furqan Mohammed Al-Asady, Department of Pharmacy, Hilla University College, Babylon 51001, Iraq.
E-mail: dr.furqan.alasady@gmail.com

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the early 1960s, *TEM*-1.[9] Among class A enzymes in Gram-negative bacteria, the *TEM-1*-encoded enzyme has been the most extensively investigated. Biofilms, by definition, are microbial communities composed of bacteria cells adapted to living in close proximity to one another, surrounded by an extracellular matrix of polymeric substances.[10,11]

Biofilms consist of living bacteria that adhere to the infected area. To survive, bacteria that produce biofilms colonize their surroundings and form biofilms on surfaces.[12] Unlike microorganisms, planktonic cells do not develop into organized clusters; instead, they grow on various surfaces.[13] Phylogeny (represented by a phylogenetic tree) illustrates the relationships among different biological groups or classifications based on genetic similarities or differences.[14] Evolutionary trees are invaluable for exploring biodiversity, genetics, ecology, and evolution among various groups of organisms.[15]

The current study aimed to focus on and address the origins of the phylogenetic tree of *P. aeruginosa* isolates recovered in Iraq, as well as to detect biofilm formation and antibiotic resistance.

Materials and Methods

Sample collection

In the study, 150 different clinical samples of various sources (burns, wounds, injuries, and diabetic patients) were collected from patients admitted to general hospitals in nine cities in Iraq (Erbil, Ninawa, Kirkuk, Diyala, Baghdad, Babylon, Muthanna, DhiQar, and Basra). The samples were obtained from patients of both genders and various age groups during the period from February 2022 until June 2022.

Isolation and identification

Samples were cultured on various culture media, including blood agar, MacConkey agar, and cetrimide agar, and then incubated at 37 °C for 24 h. Pure cultures were subsequently stored in a sterile refrigerator for future analysis. Colony morphology, including size, shape, odor, and edges, was observed. Gram stain was applied to pure cultures and examined under a microscope. Isolates were identified using morphological characteristics, biochemical tests, and the Vitek 2 Compact System (BioMerieux Marcy-l'Étoile, France), supplemented by the polymerase chain reaction technique (PCR).[16]

Detection of biofilm production

A tissue culture plate (TCP) with 96 wells and trypticase soy broth (from Himedia, India) were utilized to detect biofilm production. This method follows a semi-quantitative microtiter plate test for biofilm assay, as described by Hemati *et al*.[17] Individual wells of 96-well plates were used to cultivate *P. aeruginosa* at 37 °C in trypticase soy broth medium supplemented with 1 g of glucose. Following 24 h of growth, the plates were thoroughly washed three times with normal saline to remove free-floating bacteria. The plates were stained for 15 min at room temperature with 100 mL of 0.1% (w/v) crystal violet solution and then washed with normal saline. Subsequently, the crystal violet was removed from the wells by extracting the solution from the biofilm with 150 µl of 95% ethanol and acetone [8:2 (v/v)]. A microplate reader measured the plates at 630 nm and provided the following final results (none, weak, moderate, and strong). The results were interpreted as follows:[17]

- If OD < ODc, there is no biofilm formation.
- If ODc < OD < 2 × ODc, the bacteria were weakly adhering.
- If 2 × ODc < ODc < 4 × ODc, the bacteria were moderately attached.
- If 4 × ODc < OD, the bacteria were strongly adherent.

Extraction of DNA

The genomic DNA extraction procedure adhered to standard manufacturing and molecular identification techniques for *P. aeruginosa* (Favorgen, Taiwan).

PCR conditions and primers

Standard PCR parameters were applied to precisely identify *Pseudomonas* genes (bla_{TEM}, bla_{CTX-M}, and bla_{SHV}). The PCR primers used in this study, provided by Macrogen Company (South Korea), are detailed in Table 1.

Reaction mixture of PCR

Following the company's instructions, the PCR reaction mixture was prepared as follows: PCR Master mix (12 µL), 2 µL of forward primer, 2 µL of reverse primer, 3 µL of DNA, and the volume was completed to 25 µL with the addition of 6 µL of nuclease-free deionized water. A negative control was used, containing all the above components except DNA.

Agarose gel electrophoresis

DNA bands were photographed using a gel documentation system, as described in reference [18].

DNA sequencing

A DNA sequencing technique was performed to investigate the genetic variation of bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes of *P. aeruginosa* isolates. The PCR products were sent to Macrongen Company in Korea in an ice bag by Dalsey Hillblom Lynn. Similarity analyses were conducted using the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) analysis. bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes of *P. aeruginosa* isolates in this study were registered in the NCBI Gen Bank database with accession numbers. To obtain trimmed sequences, each data sequence was trimmed from start to finish, following typical patterns. When compared to NCBI BLAST, these

Table 1: PCR primers of Macrogen (South Korea) with conditions used in the current work

Primer		Sequence (5→3)	Amplicon size (bp)	Conditions (denaturation, annealing, and extension)	Cycle no.
TEM	F	GAGTATTCAACATT CCGTGTC	861	94°C/1 min 57°C/1 min 72°C/2 min	35
	R	TAATCAGTGAGGCACCTATCTC			
SHV	F	AAGATCCACTATCGCCAGCAG	231	94°C/30 s 64°C/1 min 72°C/2 min	35
	R	ATTCAGTTCCGTTTCCCAGCGG			
CTXm1	F	GACGATGTCACTGGCTGAGC	499	94°C/60 s 57°C/60 s 72°C/60 s	35
	R	AGCCGCCGACGCTAATACA			
P. aeruginosa	F	GGGGGATCTTCGGACCTCA	956		35
	R	TCCTTAGAGTGCCCACCCG		95°C/60 s 61°C/45 s 72°C/60 s	

F = forward primer, R = reverse primer

sequences exhibited a high degree of identity with other global sequence data. The waves produced by Scanning the sequences generated waves indicating strong and weak regions, which were subsequently trimmed, leading to increased identity with global sequences at NCBI BLAST.

Phylogenetic tree

A phylogenetic tree was designed using the Molecular Evolutionary Genetics Analysis (MEGA) 4 software program,[19] employing the neighbor-joining phylogeny tree method.[20] Evolutionary distances were calculated using the maximum composite likelihood method.[21] To assess the reliability of the trees, 1000 bootstrap replicates were generated.[15]

Statistical analysis

Data analysis was conducted using SPSS version 26, with significance set at a *P* value <0.0.

Ethical statement

The study proposal adhered to the ethical standards of the Helsinki Declaration and was approved by the local medical ethics committee, as documented by number 23 on January 21, 2022.

Results

Isolation/identification of isolates

Out of the 150 samples collected, 46 isolates (31%) of *P. aeruginosa* (named PA1–PA46) were identified. Twenty-three isolates (50%) were obtained from burns, 17 isolates (37%) were obtained from injuries, and six isolates (13%) were recovered from the feet of diabetic individuals.

Detection of biofilm formation

All isolates underwent testing using the TCP method assay. The production of biofilm by the isolates is detailed in Table 2. Results indicated that out of the 46 isolates, seven isolates (15%) (PA3, PA5, PA9, PA12, PA13, PA16, and PA19) were moderate biofilm producers, 27 isolates (59%) exhibited weak biofilm production (PA1, PA2, PA4, PA6, PA7, PA10, PA11, PA14, PA15, PA17, PA18, PA21, PA22, PA23, PA24, PA27, PA29, PA30, PA33, PA34, PA35, PA36, PA38, PA41, PA44, and PA46), and 12 isolates (26%) were non-biofilm producers. Overall, 36 isolates (74%) were reported as biofilm producers.

Resistance to different classes of antibiotics with varying grades of biofilm formation was recorded [Table 3]. The results indicated that there was no significant difference between antibiotic resistance and grades of biofilms.

In the study, 15 local isolates were chosen for DNA sequencing (PA1, PA2, PA3, PA5, PA6, PA7, PA8, PA9, PA11, PA12, PA16, PA19, PA23, PA24, and PA30), all of which were isolated from various regions of Iraq. Among these 15 selected isolates, sequence alignment revealed that 9 isolates (PA3, PA6, PA9, PA12, PA16, PA19, PA23, PA24, and PA30) exhibited identities ranging from 95% to 100% [Figures 1–9 and Table 4], good query coverage, and maximum scores when compared with other worldwide strains of *P. aeruginosa*.

Phylogenetic analysis of local and world wild strains

The dataset was cleaned of positions with gaps or missing data using the complete deletion option. Phylogenetic analysis was performed using MEGA X 10.2.4. Thirteen global taxa related to the $bla_{CTX\text{-}M}$ gene of *P. aeruginosa* were obtained from NCBI and submitted along with three local sequences to Mega X 10.2.4 software to obtain Figure 10. Similarly, 10 global taxa related to the bla_{SHV} gene of *P. aeruginosa* were obtained from NCBI and submitted with three local sequences to Mega X 10.2.4 software to obtain Figure 11. Additionally, 11 global taxa related to the bla_{TEM} gene of *P. aeruginosa* were obtained from NCBI and submitted with three

Table 2: Biofilm formation capability of *P. aeruginosa*

Isolate	Specimen type	Geographic region	OD (nm)	Grade
PA1	Burn	Muthanna	0.148	Weak
PA2	Injury	Baghdad	0.102	Weak
PA3	Diabetic foot	Ninawa	0.227	Moderate
PA4	Burn	DhiQar	0.11	Weak
PA5	Injury	Kirkuk	0.247	Moderate
PA6	Injury	Diyala	0.204	Weak
PA7	Diabetic foot	Busra	0.107	Weak
PA8	Burn	Erbil	0.105	Weak
PA9	Injury	Babylon	0.206	Moderate
PA10	Burn	Ninawa	0.149	Weak
PA11	Diabetic foot	DhiQar	0.135	Weak
PA12	Injury	Diyala	0.269	Moderate
PA13	Injury	Baghdad	0.298	Moderate
PA14	Injury	Basra	0.132	Weak
PA15	Burn	Kirkuk	0.147	Weak
PA16	Diabetic foot	Diyala	0.224	Moderate
PA17	Burn	DhiQar	0.103	Weak
PA18	Burn	Muthanna	0.112	Weak
PA19	Burn	Basra	0.203	Moderate
PA20	Burn	Basra	0.095	Non
PA21	Injury	Muthanna	0.143	Weak
PA22	Burn	Erbil	0.129	Weak
PA23	Burn	Baghdad	0.131	Weak
PA24	Burn	Baghdad	0.107	Weak
PA25	Diabetic foot	Diyala	0.095	Non
PA26	Injury	Baghdad	0.076	Non
PA27	Injury	Erbil	0.118	Weak
PA28	Burn	Babylon	0.089	Non
PA29	Diabetic foot	Basra	0.103	Weak
PA30	Injury	Kirkuk	0.108	Weak
PA31	Burn	DhiQar	0.093	Non
PA32	Burn	DhiQar	0.09	Non
PA33	Burn	Muthanna	0.137	weak
PA34	Burn	Baghdad	0.11	Weak
PA35	Burn	Muthanna	0.102	Weak
PA36	Burn	Kirkuk	0.11	Weak
PA37	Burn	Kirkuk	0.096	Non
PA38	Burn	Babylon	0.10	Weak
PA39	Injury	Baghdad	0.086	Non
PA40	Burn	Babylon	0.09	Non
PA41	Burn	DhiQar	0.126	Weak
PA42	Burn	Diyala	0.079	Non
PA43	Injury	Kirkuk	0.075	Non
PA44	Injury	Ninawa	0.114	Weak
PA45	Burn	Basra	0.095	Non
PA46	Burn	Babylon	0.167	Weak

OD = optical density

local sequences to Mega X 10.2.4 software to obtain Figure 12.

In the phylogenetic analysis of the bla_{CTX-M} gene [Figure 1], a total of 16 sequences were submitted, with three sequences representing local isolates and 13 sequences representing global isolates obtained from NCBI. These sequences were submitted to the MEGA X 10.2.4 software program to determine the phylogenic relationships among the local and global sequences. Upon submission, the sequences underwent alignment using Clustal W. Subsequently, the neighbor-joining (NJ) method with bootstrap 1000 was applied to construct the phylogenetic tree. The local sequence of *P. aeruginosa* PA3 exhibited proximity to the sequence KY792758.1 from *P. aeruginosa* strain SKGH (UAE) and Indian isolates (U130118.1 and KU130120.1).

Table 3: Correlation between biofilm grade and antibiotic resistance

Antibiotic	Biofilm grade						*P* value
	Non		Weak		Moderate		
	Number of isolates	% of resistance	Number of isolates	% of resistance	Number of isolates	% of resistance	
Aztreonam	12	100%	27	100%	7	100%	0.214
Imipinem	12	100%	27	100%	7	100%	0.214
Piperacillin	12	100%	27	100%	7	100%	0.214
Cefepime	12	100%	27	100%	7	100%	0.214
Tobramycin	12	100%	27	100%	7	100%	0.214
Netilmicin	12	100%	27	100%	7	100%	0.214
Ofloxacin	12	100%	27	100%	7	100%	0.214
Doripenem	5	41%	10	37%	2	29%	0.542
Meropenem	6	50%	9	33%	4	57%	0.76
Ceftazidim	12	100%	23	85%	5	71%	0.293
Piper./Tazo.	4	33%	8	35%	0	0%	0.471
Amikacin	9	75%	19	70%	6	86%	0.414
Gentamicin	13	100%	23	85%	6	86%	0.306
Ciprofloxacin	4	33%	11	41%	5	71%	0.605
Norfloxacin	3	25%	13	48%	2	29%	0.401
Levofloxacin	7	58%	9	33%	2	29%	0.601
Gatifloxacin	4	33%	7	26%	2	29%	0.673

DNA sequencing

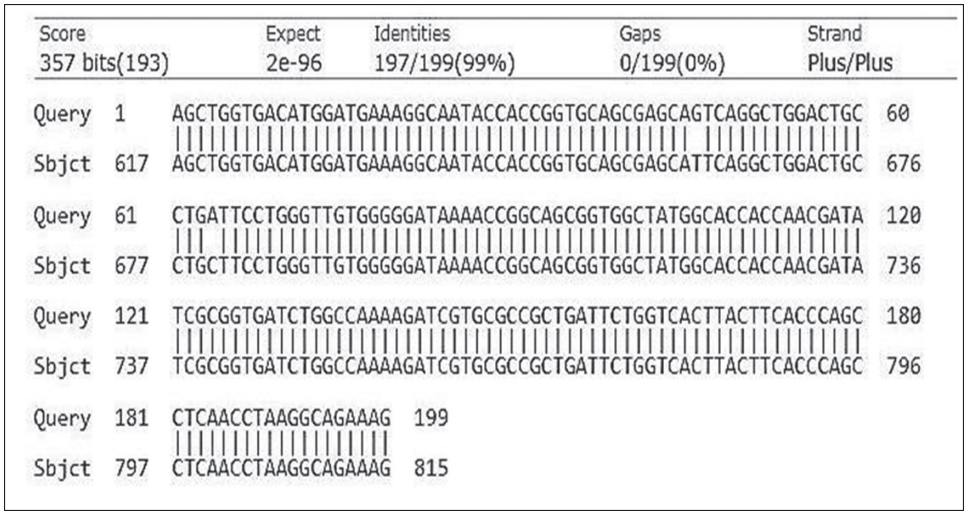

Figure 1: Basic local alignment of *P. aeruginosa* bla_{CTX-M} gene isolate PA3 with high similarity NCBI-BLAST *P. aeruginosa* strain SKGH_46 beta-lactamase (bla_{CTX-M}) gene, partial sequence (accession number: KY792758.1 in Gen Bank)

Similarly, the local sequences of *P. aeruginosa* PA6 and PA9 showed a close relationship to the sequence KR824153.1 from Indian isolates. All three local sequences were found to be distinct from Iraqi isolates (KX787848.1 and KX787849.1). In the phylogenetic analysis of the bla_{SHV} gene as shown in [Figure 12], we observed that both local sequences *P. aeruginosa* PA16 and PA19 were closely related to the sequences of Egyptian isolates (MZ700496.1 and MZ700497.1).

Additionally, the local sequence of *P. aeruginosa* PA12 was closely associated with the sequence KY640504.1 from strain E14PAMO, which was isolated in Egypt. In the phylogenetic analysis of the bla_{TEM} gene [Figure 12], the local sequences *P. aeruginosa* PA23 and PA30 were found to be closely related to each other, forming sister sequences. They were further related to the sequence MG755406.1 *P. aeruginosa* strain F35, which was isolated in Iran. In contrast, the local sequence of *P. aeruginosa* PA24 was observed to be closely associated with the sequence AY559171.1, which originated from China. These findings suggest that the phylogenic relationships among local and world strains provide valuable insights into the origin and genetic evolution of local isolates.

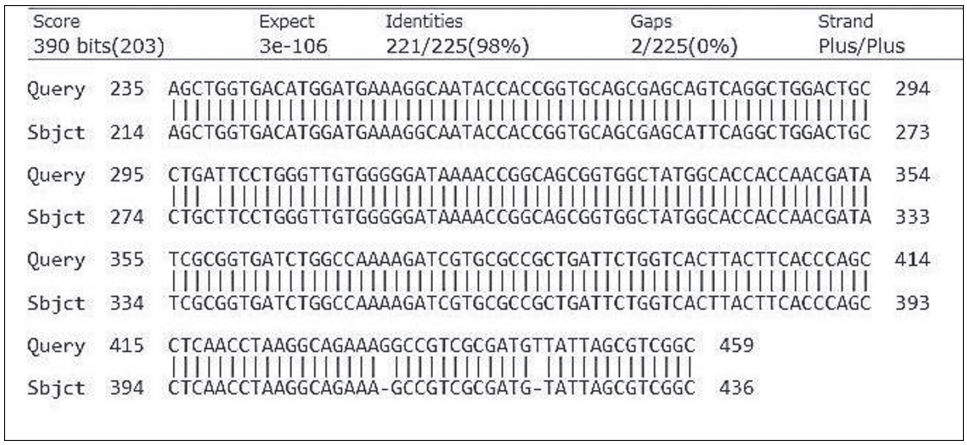

Figure 2: Basic local alignment of *P. aeruginosa* bla_{CTX-M} gene isolate PA6 with high similarity NCBI-BLAST *P. aeruginosa* strain PA137 beta-lactamase (bla_{CTX-M}) gene, partial sequence (accession number: KU139118.1 in GenBank)

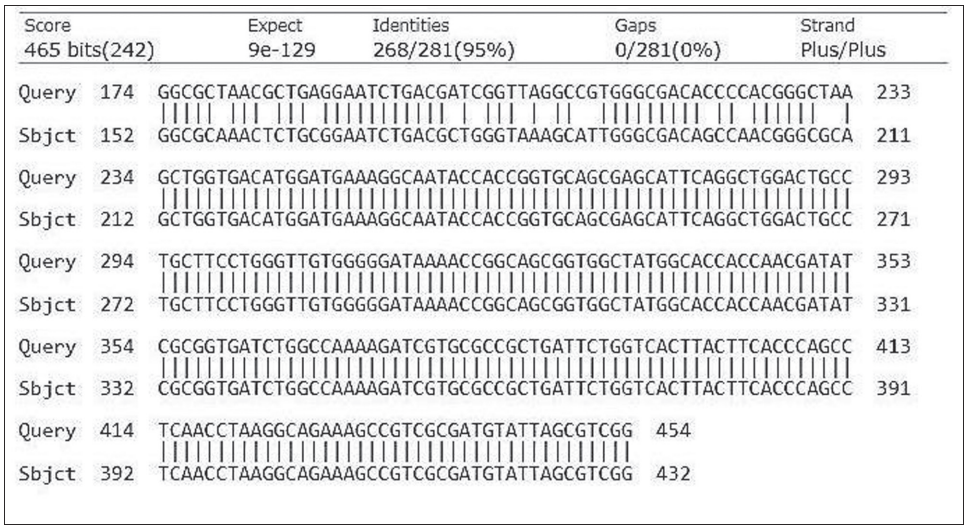

Figure 3: Basic local alignment of *P. aeruginosa* bla_{CTX-M} gene isolate PA9 with high similarity NCBI-BLAST *P. aeruginosa* strain Palg29 beta-lactamase (bla_{CTX-M}) gene, partial sequence (accession number: KU139120.1 in Gen Bank)

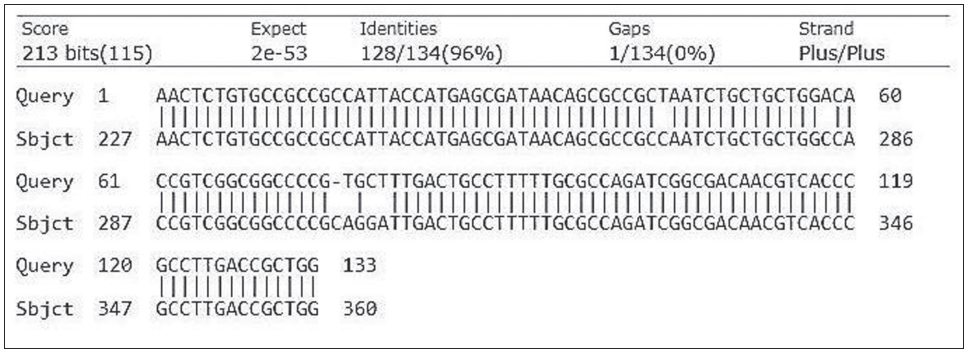

Figure 4: Basic local alignment of *P. aeruginosa* bla_{SHV} gene isolate PA12 with high similarity NCBI-BLAST *P. aeruginosa* strain E14PAMO beta-lactamase (bla_{SHV}-11) gene, partial sequence (accession number: KY640504.1 in GenBank)

Discussion

The discrepancies in results observed among different isolates in this study may be related to various reasons, including variations in the geographical areas from which the samples were collected and differences in the clinical samples themselves. These differences could lead to variations in biofilm formation capability among the isolates. Furthermore, the crucial role of bacterial cells in adhering to and responding to signaling from quorum-sensing inducers should be noted.[22]

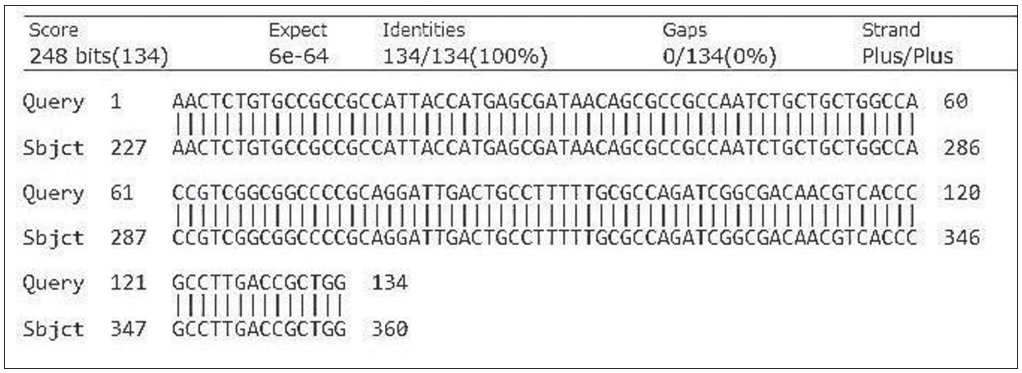

Figure 5: Basic local alignment of *P. aeruginosa* bla_{SHV} gene isolate PA16 with high similarity NCBI-BLAST *P. aeruginosa* strain E14PAMO beta-lactamase (bla_{SHV}-11) gene, partial sequence (accession number: KY640504.1 in GenBank)

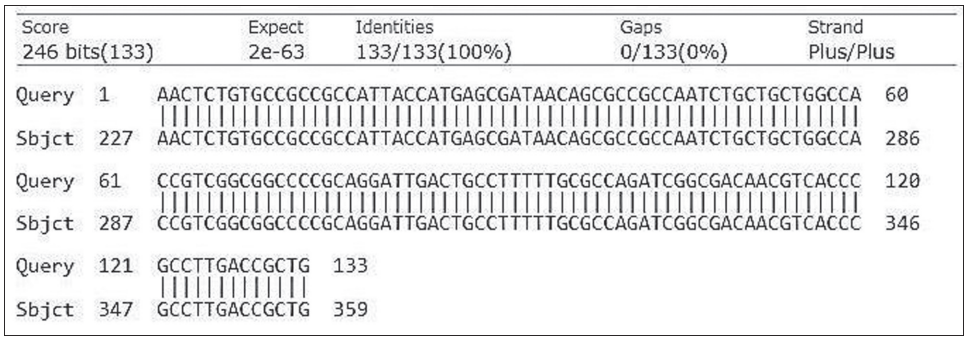

Figure 6: Basic local alignment of *P. aeruginosa* bla_{SHV} gene isolate PA19 with high similarity NCBI-BLAST *P. aeruginosa* strain E14PAMO beta-lactamase (bla_{SHV}-11) gene, partial sequence (accession number: KY640504.1 in GenBank)

Figure 7: Basic local alignment of *P. aeruginosa* bla_{SHV} gene isolate PA23 with high similarity NCBI-BLAST *P. aeruginosa* strain F35 beta-lactamase (bla_{TEM}) gene, partial sequence (accession number: MG755406.1 in GenBank)

The study noted significant variability in biofilm formation activity, with the majority of obtained isolates demonstrating biofilm-producing capabilities. This finding is considered crucial as biofilm formation is closely associated with antibiotic resistance and the chronicity of infections. These results are consistent with those reported by Karami *et al.*,[23] who found that 39 isolates (67.2%) were capable of forming biofilms, while 19 isolates (32.8%) were non-biofilm formers. In another study, a low level of biofilm formation (about 24%).[24] The TCP assay, utilized in both studies, is a rapid and straightforward method for detecting biofilm formation. It depends on the presence of a basic dye, such as Crystal Violet, which can bind to negatively charged molecules at

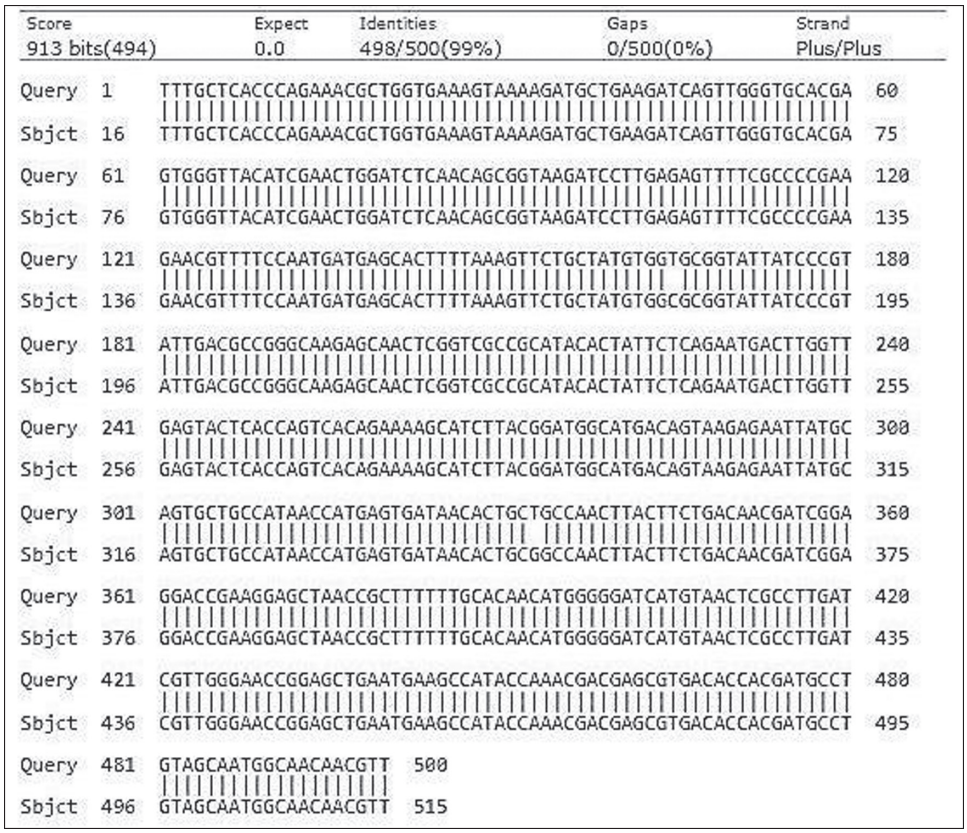

Figure 8: Basic local alignment of *P. aeruginosa* bla_{TEM} gene isolate PA24 with high similarity NCBI-BLAST *P. aeruginosa* strain F35 beta-lactamase (bla_{TEM}) gene, partial sequence (accession number: MG755406.1 in GenBank)

various sites within bacterial cells of both Gram-positive and Gram-negative bacteria.[25]

Biofilm formations are widely recognized as a significant contributor to chronic infections.[26] Many isolates of *P. aeruginosa* that are capable of producing biofilms are known to cause chronic diseases. These infections often persist and are difficult to eradicate due to the protective nature of biofilms, which provide a high level of resistance to both the immune system and antimicrobial agents.[27]

In the current study, various classes of antibiotics were used, and bacterial isolates demonstrated multiple levels of resistance to these antibiotics. The use of traditional antibiotic therapy poses challenges in eliminating biofilm bacteria due to two key reasons.[28] First, biofilms of *P. aeruginosa* are primarily composed of alginate, which forms a barrier. This barrier not only triggers tolerance to various host immune mechanisms but also confers resistance to different classes of antimicrobials. As a result, biofilms not only enhance attachment to epithelial cells but also contribute to treatment failure. Additionally, biofilms confer the advantage of continuous colonization of both living and even non-living surfaces.[29] The second reason is that bacteria within biofilms are typically slow-growing or even non-growing. This presents a challenge for antibiotics, as they are most effective against bacteria that are actively growing and dividing. The results of this study are consistent with those of another study.[30] It has been demonstrated that beta-lactam antibiotics can induce or increase the production of biofilm volume and increase alginate production in *P. aeruginosa* biofilms. This phenomenon can promote genetic exchange and the spread of antibiotic resistance genes.[31]

In local studies conducted in Iraq, the role of biofilm-forming isolates of *P. aeruginosa* in the development of resistance to various classes of antibiotics, as well as resistance to heavy metals, has been reported.[32,33]

The MEGA program is desktop software designed to compare homologous gene sequences from various species or multi-gene families. Its primary focus is on inferring evolutionary links and patterns of DNA and protein evolution. MEGA features a range of valuable tools for assembling sequence datasets from files or web-based repositories, as well as tools for visualizing results through interactive phylogenetic trees and evolutionary distance matrices.[34] The initial step in the analysis involved aligning all sequences from three genes in this study with other worldwide references using MEGA X 10.2.4's Clustal W program. This program demonstrated a high degree of similarity with all worldwide sequences, including those used in this study. The results obtained

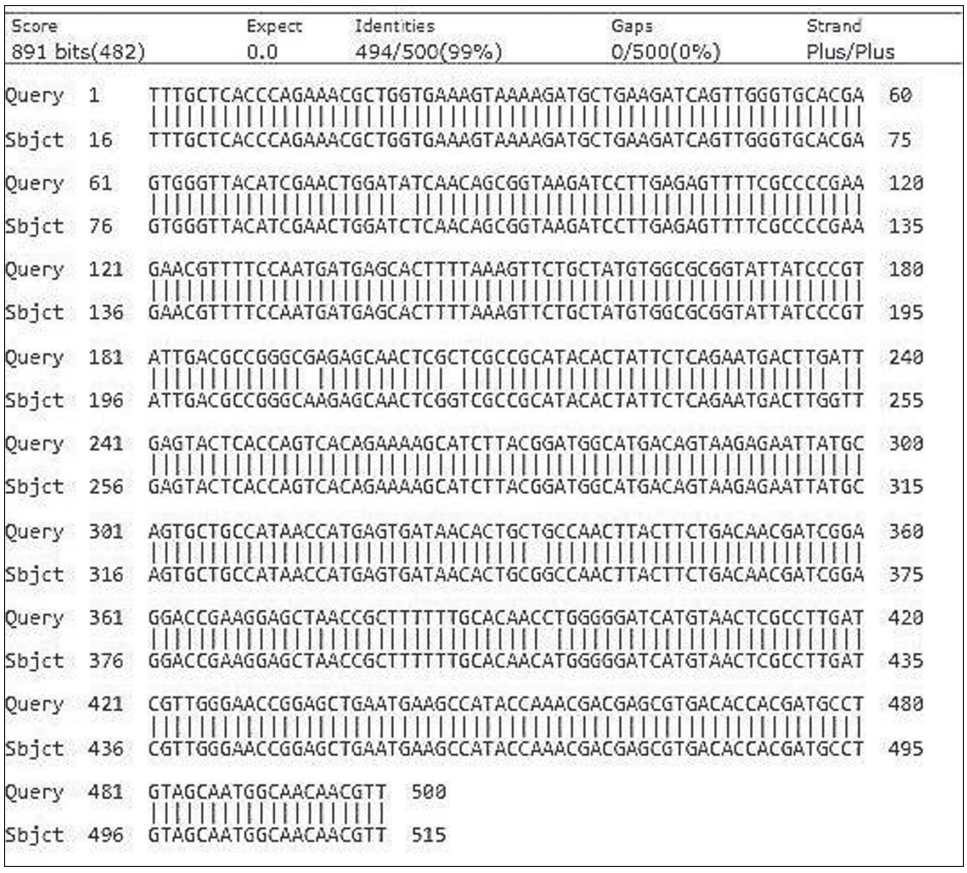

Figure 9: Basic local alignment of *P. aeruginosa* bla_{TEM} gene isolate PA30 with high similarity NCBI-BLAST *P. aeruginosa* strain F35 beta-lactamase (bla_{TEM}) gene, partial sequence (accession number: MG755406.1 in Gen Bank)

Table 4: Alignment results of *P. aeruginosa* isolates with reference isolates retrieved from NCBI

Local isolate	Reference of the isolate with the highest percentage similarity (%)			
	Gene	Accession no.	Similarity (%)	Origin
P. aeruginosa PA3	bla_{CTX-M}	KY792758.1	99	UAE
P. aeruginosa PA6	bla_{CTX-M}	KU139118.1	98	India
P. aeruginosa PA9	bla_{CTX-M}	KU139120.1	95	India
P. aeruginosa PA12	bla_{SHV}	KY640504.1	96	Egypt
P. aeruginosa PA516	bla_{SHV}	KY640504.1	100	Egypt
P. aeruginosa PA619	bla_{SHV}	KY640504.1	100	Egypt
P. aeruginosa PA23	bla_{TEM}	MG755406.1	99	Iran
P. aeruginosa PA24	bla_{TEM}	MG755406.1	99	Iran
P. aeruginosa PA30	bla_{TEM}	MG755406.1	99	Iran

from Clustal W alignment were significant as they were directly utilized in the design of phylogenetic trees.

In this study, the NJ approach, which is a simplified version of the minimal evolution method, was used to determine the close relationship between world and local sequences. Unlike some other methods, the NJ method does not require the assumption of a constant rate of evolution, resulting in an un-rooted tree. However, an outgroup taxon is necessary to find the root.[35] Furthermore, Mohammed *et al.*[36] demonstrated that the phylogenetic tree of *CTX-M-9* gene sequences in *Escherichia coli* strains isolated from ZU hospitals and published homologous sequences in GenBank revealed varying degrees of dissimilarity/similarity between strains. Moreover, many unique sequences were observed in the Egyptian strain, which exhibited similarity to strains from Russia and Australia but not to those from Japan.

According to the phylogenetic tree, the *SHV* gene encoded for *Klebsiella pneumonia* exhibited a compatibility range

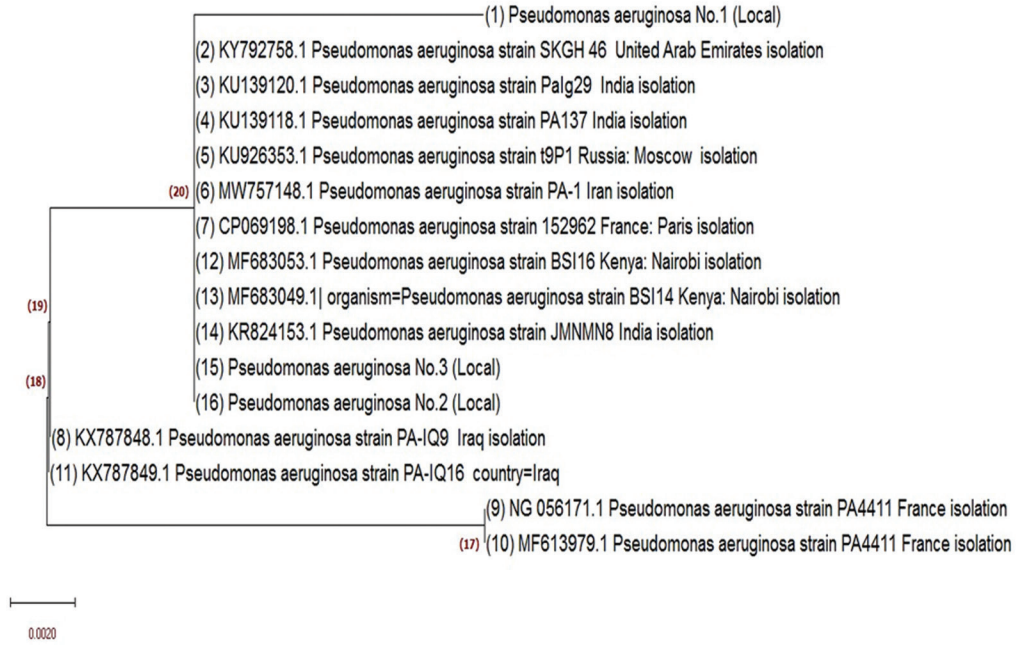

Figure 10: Phylogenic tree of partial sequences of $bla_{CTX\text{-}M}$ gene from local and global sequences using neighbor-joining bootstrap 1000 tree figure. Evolutionary relationships of 16 taxa. PA3 (No. 1), PA6 (No. 2), and PA9 (No. 3) represent the local isolates

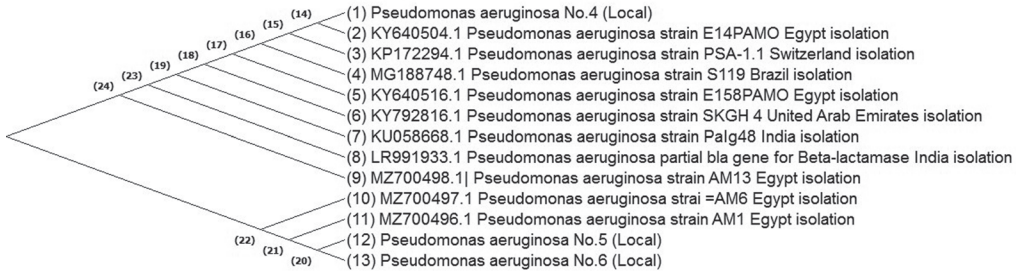

Figure 11: Phylogenic tree of partial sequences of $bla_{CTX\text{-}M}$ gene from local and global sequences using neighbor-joining bootstrap 1000 tree figure. Evolutionary relationships of 13 taxa. PA12 (No. 4), PA16 (No. 5), and PA19 (No. 6) represent local isolates

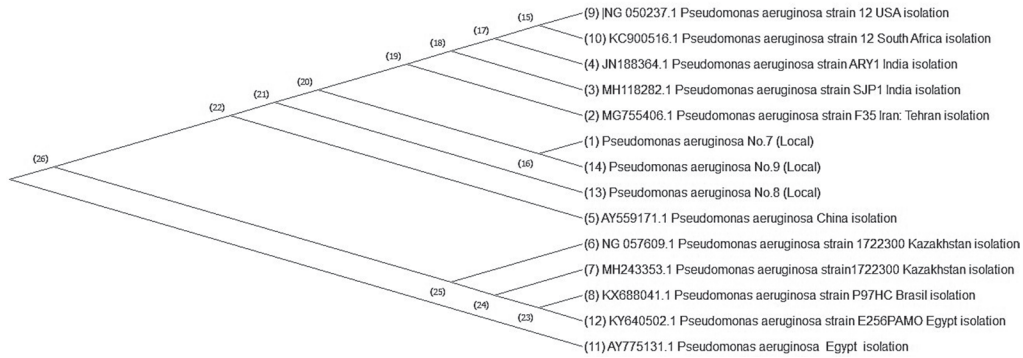

Figure 12: Phylogenic tree of partial sequences bla_{TEM} gene from local and global sequences using neighbor-joining bootstrap 1000 tree figure. Evolutionary relationships of 14 taxa. PA23 (No. 7), PA24 (No. 8), and PA30 (No. 9) represent local isolates

of 98%, while the *SHV* gene encoded for *E. coli* showed a compatibility range of 99%. However, the *SHV* gene encoded for *P. aeruginosa* isolates displayed variations in the compatibility range with different countries. It showed a compatibility range of 99% in Brazil, Egypt, United Arab Emirates, India, Japan, Tunisia, France, and Switzerland, followed by Greece (98%). The compatibility range dropped to 74% in the USA and Brazil, specifically in Belo Horizonte, and further decreased to 71% in Colombia.[37,38]

Conclusion

This study concluded that *P. aeruginosa* harbors virulence factors, including biofilm formation, which varies according to geographic areas and is strongly related to antibiotic resistance and the chronicity of infections. Antimicrobial susceptibility testing is essential for minimizing and controlling bacterial resistance, thereby addressing life-threatening infections effectively. Moreover, numerous isolates of *P. aeruginosa* have been identified from various areas of Iraq, displaying genetic similarities with isolates from other countries according to phylogenetic analysis.

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Conflict of interest

No conflict of interest.

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