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GENETIC DIVERSITY AND ANTIBIOTIC RESISTANCE PATTERNS OF PSEUDOMONAS AERUGINOSA ISOLATES FROM IRAQI HOSPITALS

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Abstract: Forty-six isolates of Pseudomonas aeruginosa were isolated and identified from 150 samples of different patients who were admitted to the general hospitals in nine cites of Iraq during the period of Sep./2021 to Jan./2022. The isolates were given names as PA1 to PA46. The biofilm formation by these isolates, using microtiter plates assay and the correlation between biofilm grades and antibiotics resistance were also studied. It was found that 34 isolates were able to produce biofilm. To determinate the sites of blaTEM, blaCTX-M, and blaSHV genes on the genome of the isolates, the whole genomic DNA of nine isolates, PA1 to PA9 was extracted and the sequencing of these genes was achieved. The matching of the isolates with NCBI-Gen bank global Pseudomonas strains, showed that one isolate (PA1) was related to UAE, two isolates (PA2 and PA3) were related to India, three isolates (PA4, PA5 and PA6) were related to Egypt and also three isolates (PA7, PA8 and PA9) were related to Iran. Hence, the variable frequencies in the sequencing of blaTEM, blaCTX-M, and blaSHV genes need further studies for creating the genetic diversity map of P. aeruginosa.

Keywords: P. aeruginosa, multidrug-resistance, DNA sequencing, virulence factors, biofilm, phylogenetic tree and phylogenetic origin.

1. Introduction

P. aeruginosa is opportunistic pathogen, that cause various diseases and frequently resistant to many commonly used antibiotics. This bacterium accounts for 10-15% of the nosocomial infections worldwide and is considered the third most common organism associated with hospital-acquired infections. Extended spectrum β-lactamases (ESBLs), which produced by multidrug-resistant (MDR) P. aeruginosa, are a critical problem that demands efficient infection management strategies to break their spread [1].

The prevalence of clinical isolates varies widely around the world and changes fast over time, it is a leading to many debilitating infections, including wound infections, pneumonia, and keratitis [2]. The numerous resistance mechanisms, particularly against last-resort drugs, make treating associated infections challenging, resulting in treatment failure, prolonged hospitalization, and high morbidity and mortality [3]. P. aeruginosa has

ability to form biofilms on medical devices, which consider one of its virulence factors [4]. [5] reported that MDR strains of P. aeruginosa has become the common problem, especially in the chronic cases because of the development in the resistance mechanisms of antibiotics. P. aeruginosa, develop a group of enzymes known as ESBLs, which are capable of hydrolyzing antimicrobial drugs such as penicillins, cephalosporins, monobactams, and carbapenems, and causing resistance to them [6].

The most common ESBL genes found in P. aeruginosa are SHV, CTX-M, and TEM kinds, which all belong to the sulfhydryl variable (SHV) family [1]. A phylogenetic tree, also known as an evolutionary tree or phylogeny, is used in a branch of biology that analyzes morphological data matrices and molecular sequencing data to identify how various groups of animals have evolved over time. It has been used to explore the biodiversity, evolution, genetics, and ecology among groups of organisms [7]. Hence, the present work was focused on the phylogenetic origins of P. aeruginosa isolates in Iraq.

2. Materials and Methods

Collection of Samples: One hundred and fifty samples of wound, diabetic foot and burn infections of both genders and different ages of patients were collected from different cities of Iraq during the period of September 2021 to January 2022.

Isolation and Identification: The isolation and identification were carried out depending on [1].

Detection of Biofilm Production: The biofilm prodauction of the isolates were determined according to [8].

DNA Extraction: Whole genomic DNA was extracted according to the manufacturer standard for *P. aeruginosa* molecular identification (Favorgen, Taiwan).

PCR Primers and Conditions: PCR cycling thermal program parameters were used for detection of *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$ genes. The Macrogen (South Korea) manufactured of PCR primers are shown in Table 1.

Table 1: PCR primers and their conditions.

Primer		Sequence (5----------›3)	Amplicon size (bp)	Conditions (D, A and E)	Cycle No.	Source
TEM	F	GAGTATTCAACATT CCGTGTC	861	94˚C/1 min. 57˚C/1 min. 72˚C/2 min.	35	(Bokaeian *et a*l., 2015)
	R	TAATCAGTGAGGCAC-CTATCTC				
SHV	F	AAGATCCACTATCGCCAG-CAG	231	94˚C/30 sec 64˚C/1 min. 72˚C/2 min.	35	
	R	ATTCAG-TTCCGTTTCCCAGCGG				
CTXm1	F	GACGATGTCACTGGCTGAGC	499	94˚C/1min. 57˚C/1min. 72˚C/1min.	35	
	R	AGCCGCCGACGCTAATACA				
P.aeru	F	GGGGGATCTTCGGACCTCA	956	95˚C/1min 61˚C/45sec 72˚C/1min	35	(Spilker *et al*., 2004)
	R	TCCTTAGAGTGCCCACCCG				

Abbreviations: D, Denaturation; A, Annealing; E, Extension; F, Forward primer; R, Reverse primer.

Preparation of PCR Reaction Mixture: The reaction mixture of PCR was performed according to [1].

Electrophoresis: The amplified products of PCR were run on 1% of horizontal agarose gel at 75 volts, stained with red safe dye for 1 hour. 5 µl of each product of the isolates plus 1 µl of loading dye were loaded in the wells of the gel. 100-1500 bp DNA ladder (Promega, USA) was used to detect the size of amplified gene fragments. The DNA bands were imaged using a gel documentation system (Biometra-Germany) as described by [9].

DNA Sequencing: To study the genetic variation of *P. aeruginosa* isolates, DNA sequencing was performed. The PCR products were sent to Macrongen company in Korea in ice bag by DHL. The homology sequence identity and the mutation analysis were conducted using NCBI BLAST analysis. *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes of *P. aeruginosa* isolates of the current study were registered in NCBI-Gen Bank data base with accession numbers (Table 5).

Phylogenetic Tree: The phylogenetic tree was designed using the neighbor method with the help of MEGA4 software program and the neighbor-joining phylogeny tree [10]. The evolutionary distances are computed using the maximum composite likelihood method [7] and the reliability of the trees were determined by 1000 data set bootstrap resembling [11].

3. Results and Discussion:

One hundred and fifty clinical specimens of burns, injuries and feet of diabetics who were admitted to the general hospitals in nine cites of Iraq which included Erbil, Ninawa, Kirkuk, Diyala, Baghdad, Babylon, Muthanna, DhiQar and Basra were collected. Forty-six isolates of *P. aeruginosa* (PA1 to PA46) were isolated from the specimens of these isolates, 23 (50%) were isolated from burns, 17 (37 %) were isolated from injuries, and 6 (13 %) were isolated from feet of diabetics. The isolates were identified depending on traditional methods (morphological features of the colonies and the cells, biochemical tests, Vitek 2 compact) and finally, the confirmation was achieved via PCR. Thirty isolates were tested for biofilm formation using tissue culture plate (TCP) assay, as it is a semi-quantitative microtiter plate. The interpretation of biofilm formation was done and the biofilm capacity of *P. aeruginosa* was demonstrated in Table 2. The results showed that out of 46 isolates only 7 (%14) isolates (PA3, PA5, PA9, PA12, PA13, PA16, PA19) were moderate positive to form biofilm, the weakly positive isolates were 27 (59%) (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 the negative isolate were 12 (27 %) as shown in Figure 1.

Table 2: Biofilm forming capacity of *P. aeruginosa* isolates.

Isolate No.		Specimen type	Geographic region	OD (nm)	Grade
P. aeruginosa	**PA1**	Burn	Muthanna	0.148	weak
P. aeruginosa	**PA2**	Injury	Baghdad	0.102	weak
P. aeruginosa	**PA3**	Diabetic Foot	Ninawa	0.227	moderate
P. aeruginosa	**PA4**	Burn	DhiQar	0.11	weak
P. aeruginosa	**PA5**	Injury	Kirkuk	0.247	moderate
P. aeruginosa	**PA6**	Injury	Diyala	0.204	weak
P. aeruginosa	**PA7**	Diabetic Foot	Busra	0.107	weak
P. aeruginosa	**PA8**	Burn	Erbil	0.105	weak
P. aeruginosa	**PA9**	Injury	Babylon	0.206	moderate
P. aeruginosa	**PA10**	Burn	Ninawa	0.149	weak
P. aeruginosa	**PA11**	Diabetic Foot	DhiQar	0.135	weak
P. aeruginosa	**PA12**	Injury	Diyala	0.269	moderate

P. aeruginosa	**PA13**	Injury	Baghdad	0.298	moderate
P. aeruginosa	**PA14**	Injury	Basra	0.132	weak
P. aeruginosa	**PA15**	Burn	Kirkuk	0.147	weak
P. aeruginosa	**PA16**	Diabetic Foot	Diyala	0.224	moderate
P. aeruginosa	**PA17**	Burn	DhiQar	0.103	weak
P. aeruginosa	**PA18**	Burn	Muthanna	0.112	weak
P. aeruginosa	**PA19**	Burn	Basra	0.203	moderate
P. aeruginosa	**PA20**	Burn	Basra	0.095	non
P. aeruginosa	**PA21**	Injury	Muthanna	0.143	weak
P. aeruginosa	**PA22**	Burn	Erbil	0.129	weak
P. aeruginosa	**PA23**	Burn	Baghdad	0.131	weak
P. aeruginosa	**PA24**	Burn	Baghdad	0.107	weak
P. aeruginosa	**PA25**	Diabetic Foot	Diyala	0.095	non
P. aeruginosa	**PA26**	Injury	Baghdad	0.076	non
P. aeruginosa	**PA27**	Injury	Erbil	0.118	weak
P. aeruginosa	**PA28**	Burn	Babylon	0.089	non
P. aeruginosa	**PA29**	Diabetic Foot	Basra	0.103	weak
P. aeruginosa	**PA30**	Injury	Kirkuk	0.108	weak
P. aeruginosa	**PA31**	Burn	DhiQar	0.093	non
P. aeruginosa	**PA32**	Burn	DhiQar	0.09	non
P. aeruginosa	**PA33**	Burn	Muthanna	0.137	weak
P. aeruginosa	**PA34**	Burn	Baghdad	0.11	weak
P. aeruginosa	**PA35**	Burn	Muthanna	0.102	weak
P. aeruginosa	**PA36**	Burn	Kirkuk	0.11	weak
P. aeruginosa	**PA37**	Burn	Kirkuk	0.096	non
P. aeruginosa	**PA38**	Burn	Babylon	0.10	weak
P. aeruginosa	**PA39**	Injury	Baghdad	0.086	non
P. aeruginosa	**PA40**	Burn	Babylon	0.09	non
P. aeruginosa	**PA41**	Burn	DhiQar	0.126	weak
P. aeruginosa	**PA42**	Burn	Diyala	0.079	non
P. aeruginosa	**PA43**	Injury	Kirkuk	0.075	non
P. aeruginosa	**PA44**	Injury	Ninawa	0.114	weak
P. aeruginosa	**PA45**	Burn	Basra	0.095	non
P. aeruginosa	**PA46**	Burn	Babylon	0.167	weak

The biofilms were important to protect the bacteria against antimicrobial agents, Therefore, the discrepancy of results between different isolates in this study may be attributed to many factors such as, the type of clinical specimens from which the isolates were obtained and also the differences of isolates capability to form biofilm. The primary number of cells that succeeded in adherence and the differences of quality and quantity of autoinducers (quorum sensing signaling molecules) that were produced from each isolate may also play an essential and an important role.

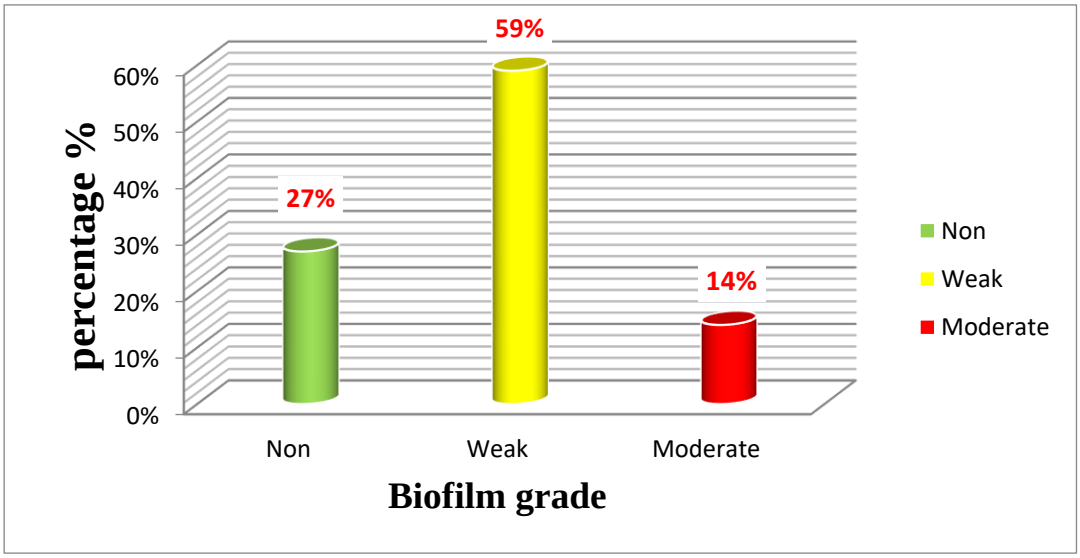

Figure 1: Biofilm formation grade of *P. aeruginosa* isolates

It was observed in Figure 1 that a variable activity in biofilm formation was found. This result has an agreement with the result of [4] who noted that *P. aeruginosa* isolates exhibited variable biofilm productions. TCP assay was a simple and rapid method to quantify biofilm formation of different bacterial strains. Crystal violet is a basic dye known to bind to negatively charged molecules on the cell surface as well as nucleic acids and polysaccharides, and therefore gives an overall measure of the whole biofilm. It has been used as a standard technique for rapidly accessing cell attachment and biofilm formation in a range of Gram positive and Gram-negative bacteria [12]. In the current study regarding to the percentages between biofilm formation and antibiotic resistance, it was showed that not all the isolates were 100 % for biofilm formation and multi-drug resistant (Table 3. The biofilm risk originated from the fact that, it is the main driver of persistence of chronic infections. Many of *P. aeruginosa* that caused chronic infections have been linked to the biofilm mode of growth and such infections are difficult to eradicate because bacteria in biofilms have a higher tolerance against antimicrobial agents than their planktonic counterparts.

Table 3: The grade and percentage of biofilm formation of P. aeruginosa isolates.

Biofilm grade	Isolate No.	%
Non	13	28
Weak	27	59
Moderate	6	13
Total	46	100

High resistance to the antibiotics with different grades of biofilm formation was recorded in this study (Table 4), where the difference between antibiotic resistance and the grade of biofilms was no significant difference. This was a reason or evidence that once a biofilm exists in any grade, it is sufficient to resist different lifetime antibiotics and this was what distinguishes of *P. aeruginosa* and makes them multi-drug resistant.

Table 4: Correlation between biofilm grade and antibiotic resistance of P. aeruginosa isolates.

Antibiotic	Biofilm grade						P. value
	Non		Weak		Moderate		
	No.	%	No.	%	No.	%	
Aztreonam	13	28	27	59	6	13	0.214
Imipinem	13	28	27	59	6	13	0.214
Piperacillin	13	28	27	59	6	13	0.214
Cefepime	13	28	27	59	6	13	0.214
Tobramycin	13	28	27	59	6	13	0.214
Netilmicin	13	28	27	59	6	13	0.214
Ofloxacin	13	28	27	59	6	13	0.214
Doripenem	5	29	10	59	2	12	0.542
Meropenem	6	32	9	47	4	21	0.76
Ceftazidim	12	30	23	57	5	13	0.293
Piper./Tazo.	4	33	8	67	0	0	0.471
Amikacin	9	26	19	56	6	18	0.414
Gentamicin	13	31	23	55	6	14	0.306
Ciprofloxacin	4	20	11	55	5	25	0.605
Norfloxacin	3	17	13	72	2	11	0.401
Levofloxacin	7	39	9	50	2	11	0.601
Gatifloxacin	4	32	7	53	2	15	0.673

There are two key reasons why the use of traditional antibiotic therapy makes biofilm bacteria hard to eliminate. Biofilm polysaccharide which is also referred to as slime, is a polymeric conglomeration generally composed of proteins and polysaccharides [4]. The extracellular exopolysaccharide of biofilms of *P. aeruginosa* is mainly composed of alginate. It has been stressed that matrix provides a barrier leading to enhance resistance to host defense mechanism as well as to antibiotics causing treatment failure and also promote adherence to epithelial cells. Biofilm formation provides bacteria with a means of persistently colonizing either living or inert surfaces within a human host [13]. The results of the current study were agreed with [14] study. Beta-lactam antibiotics have been shown to induce or increase production of biofilm volume and increase alginate production in *P. aeruginosa* biofilms, which increase the genetic exchange and the spread of antibiotic resistance genes.

To obtain a trimmed sequence, each data sequence was trimmed from beginning to ending, according to normal waves. When compared with NCBI- Blast, this sequence has a high level of identity to other global sequence data. The waves produced by scanning the sequences indicate the strong and weak regions of the sequences, which are then trimmed, resulting in increased identity with global sequences at NCBI-Blasting. The results of nucleotide sets are checked and confirmed by using NCBI–Basic Local Alignment Search Tool (BLAST analysis)-nucleotide Blast-Search a nucleotide database using a nucleotide query online, which was a perfect program and gave the exact results of identity percentage with other world strains. Sequence alignment must be performed using *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes of *P. aeruginosa* sequences databases information recorded in Gen Bank to find identity and similarity score degrees of genes and compared with the local isolates of this study. The results of sequences alignment of the nine local isolates (PA1 to PA9, isolated from different regions of Iraq, Table 2 and 6) showed identity ranging from 95% to 100% (Figure. 2 to 10 and Table 5), good query cover, and max score with other world strains of *P. aeruginosa*.

Table 5: Alignment results of P. aeruginosa isolates with reference isolates retired from NCBI.

Isolate No.	Reference of the isolate with highest percentage similarity (%)			
	Gene	Accession No.	Similarity (%)	Origin
P. aeruginosa PA1	*bla*$_{CTX-M}$	KY792758.1	99 %	UAE
P. aeruginosa PA2	*bla*$_{CTX-M}$	KU139118.1	98 %	India
P. aeruginosa PA3	*bla*$_{CTX-M}$	KU139120.1	95 %	India
P. aeruginosa PA4	*bla*$_{SHV}$	KY640504.1	96 %	Egypt
P. aeruginosa PA5	*bla*$_{SHV}$	KY640504.1	100 %	Egypt
P. aeruginosa PA6	*bla*$_{SHV}$	KY640504.1	100 %	Egypt
P. aeruginosa PA7	*bla*$_{TEM}$	MG755406.1	99 %	Iran
P. aeruginosa PA8	*bla*$_{TEM}$	MG755406.1	99 %	Iran
P. aeruginosa PA9	*bla*$_{TEM}$	MG755406.1	99 %	Iran

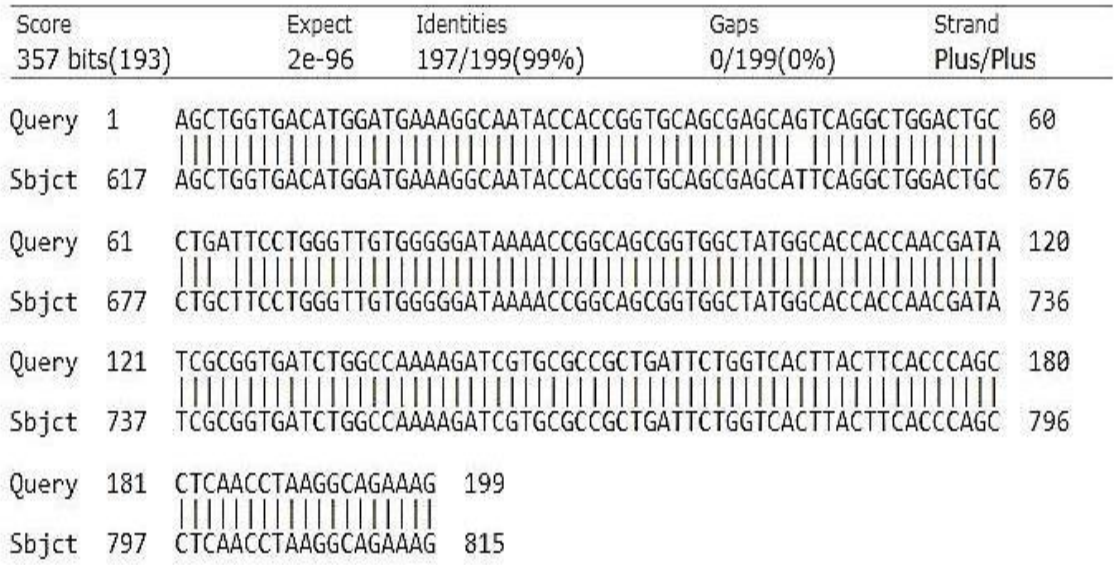

Figure 2: Basic local alignment of P. aeruginosa blaCTX-M gene of the isolate PA1 with high similarity NCBI-BLAST of P. aeruginosa strain SKGH_46 B-lactamase (blaCTX-M) gene, partial sequence (accession number: KY792758.1 in Gen Bank).

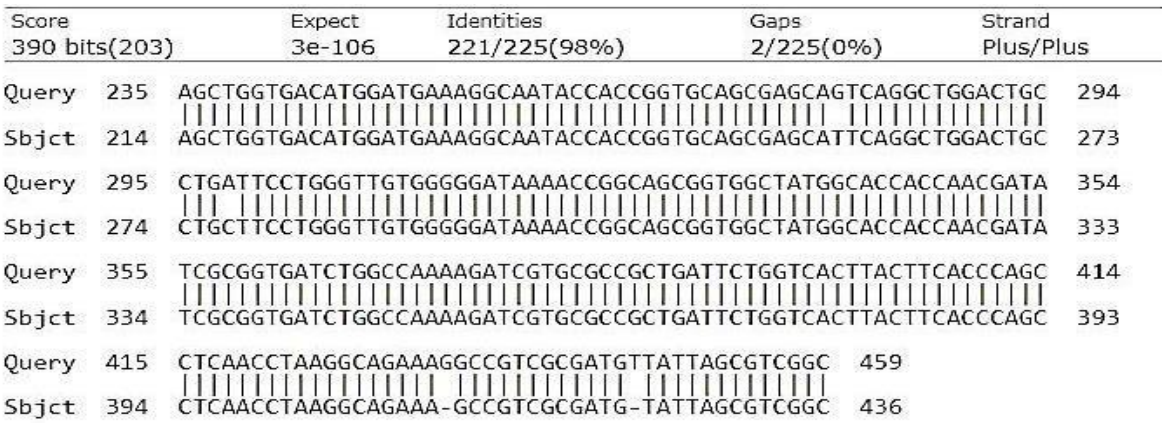

Figure 3: Basic local alignment of P. aeruginosa blaCTX-M gene of the isolate PA2 with high similarity NCBI-BLAST of P. aeruginosa strain PA137 B-lactamase (blaCTX-M) gene, partial sequence (accession number: KU139118.1 in GenBank).

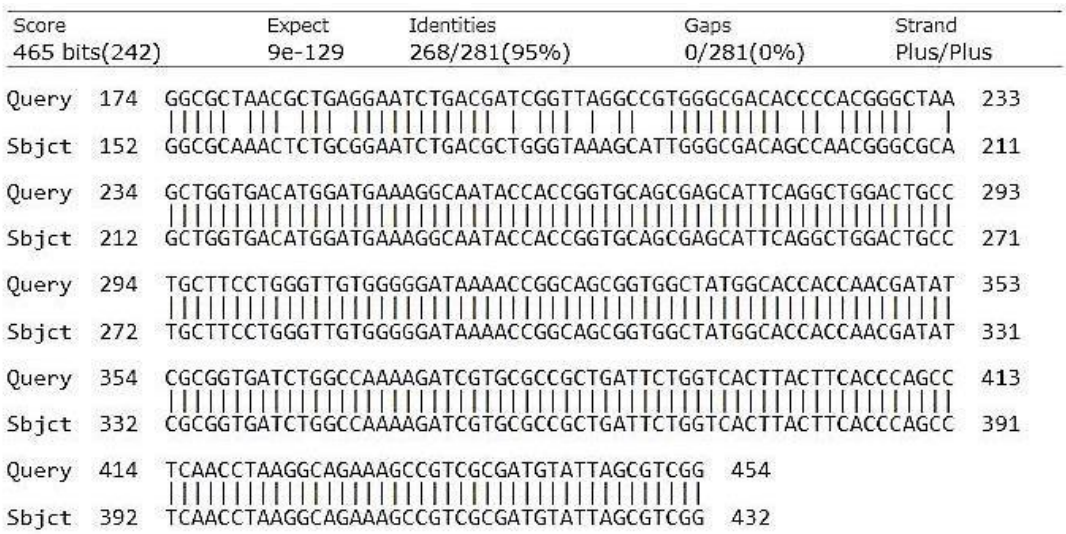

Figure 4: Basic local alignment of P. aeruginosa blaCTX-M gene of the isolate PA3 with high similarity NCBI-BLAST of P. aeruginosa strain Palg29 B-lactamase (blaCTX-M) gene, partial sequence (accession number: KU139120.1 in Gen Bank).

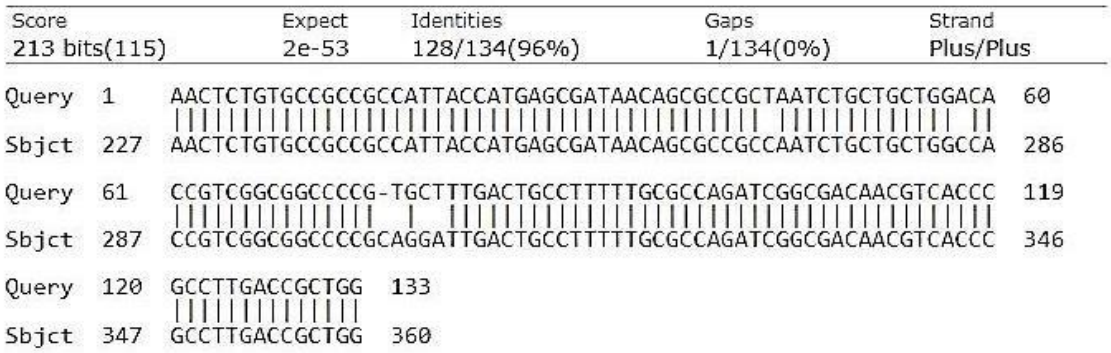

Figure 5: Basic local alignment of P. aeruginosa blaSHV gene of the isolate PA4 with high similarity NCBI-BLAST of P. aeruginosa strain E14PAMO B-lactamase (blaSHV -11) gene, partial sequence (accession number: KY640504.1 in GenBank).

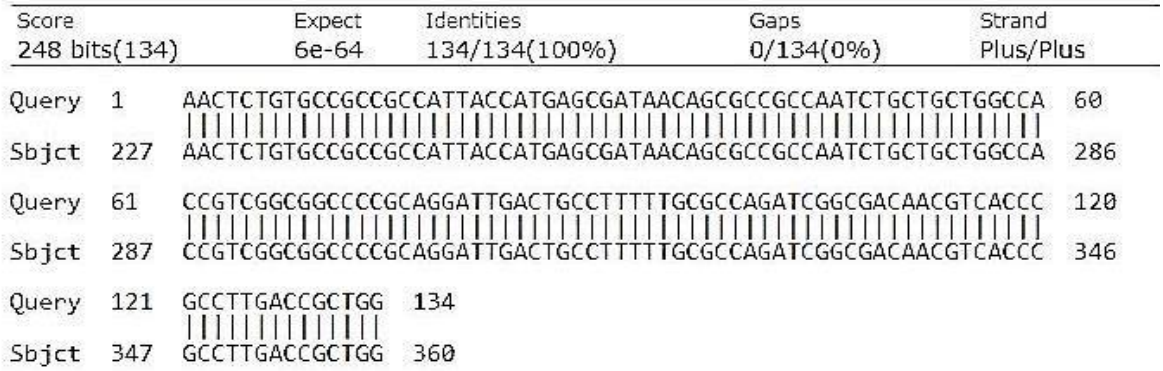

Figure 6: Basic local alignment of P. aeruginosa blaSHV gene of the isolate PA5 with high similarity NCBI-BLAST of P. aeruginosa strain E14PAMO B-lactamase (blaSHV -11) gene, partial sequence (accession number: KY640504.1 in GenBank).

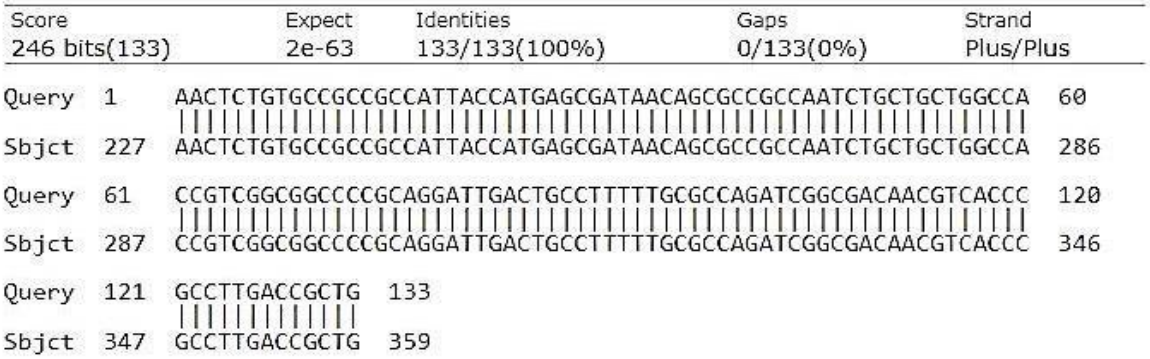

Figure 7: Basic local alignment of P. aeruginosa blaSHV gene of the isolate PA6 with high similarity NCBI-BLAST of P. aeruginosa strain E14PAMO B-lactamase (blaSHV -11) gene, partial sequence (accession number: KY640504.1 in GenBank).

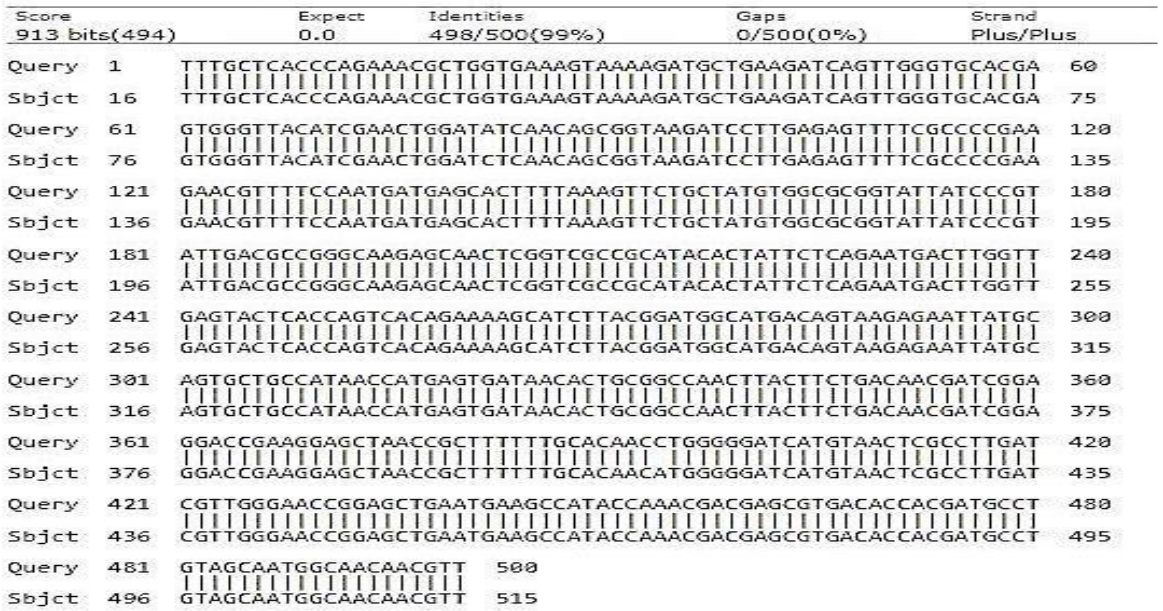

Figure 8: Basic local alignment of P. aeruginosa blaTEM gene of the isolate PA7 with high similarity NCBI-BLAST of P. aeruginosa strain F35 B-lactamase (blaTEM) gene, partial sequence (accession number: MG755406.1in GenBank).

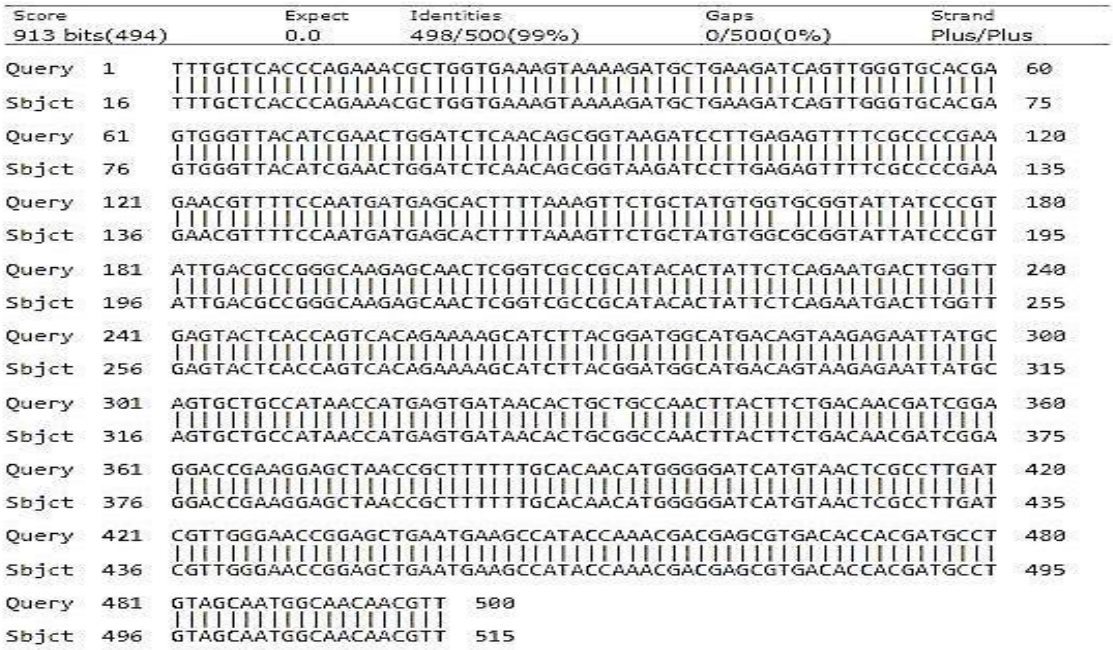

Figure 9: Basic local alignment of P. aeruginosa blaTEM gene of the isolate PA8 with high similarity NCBI-BLAST of P. aeruginosa strain F35 B-lactamase (blaTEM) gene, partial sequence (accession number: MG755406.1in GenBank).

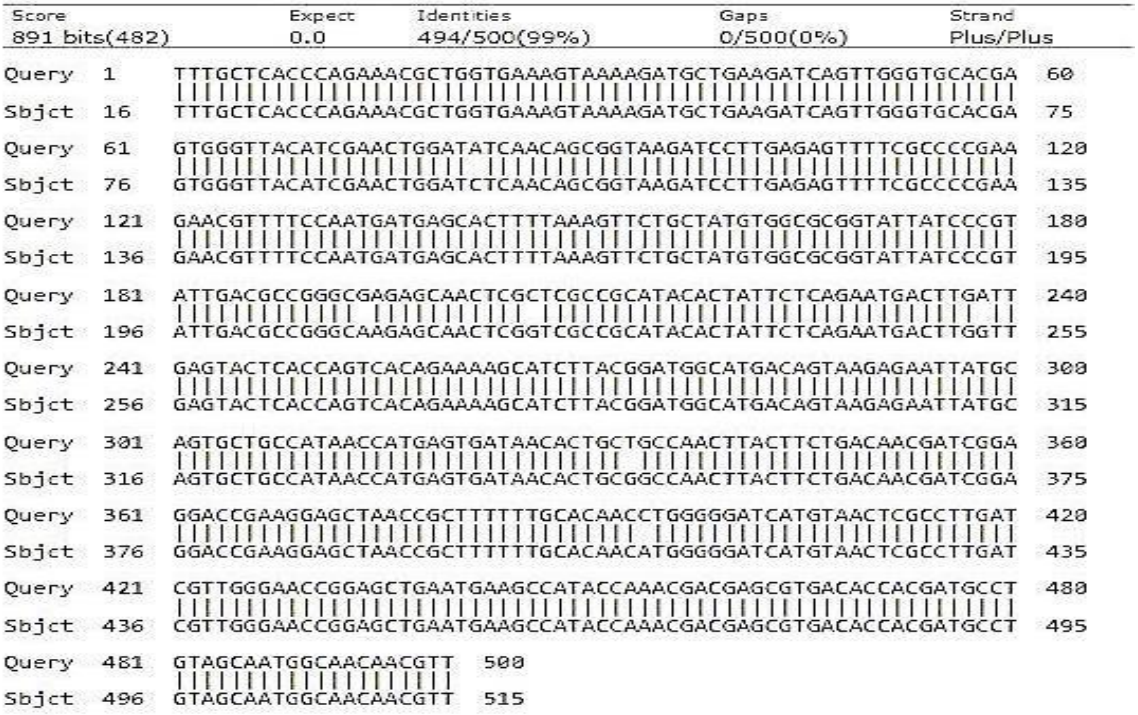

Figure 10: Basic local alignment of P. aeruginosa blaTEM gene of the isolate PA9 with high similarity NCBI-BLAST of P. aeruginosa strain F35 B-lactamase (blaTEM) gene, partial sequence (accession number: MG755406.1 in Gen Bank).

The phylogenetic tree was drawn to scale with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The dataset was cleansed of positions with gaps or missing data (Complete deletion option). MEGA X 10.2.4 is used to perform phylogenetic analysis. Thirteen global taxa about *bla*$_{CTX-M}$ gene of *P. aeruginosa* were downloaded from NCBI and submitted with 3 local sequences to Mega X

10.2.4 software to obtain Figure 11. Ten global taxa about *blaSHV* gene of *P. aeruginosa* were downloaded from NCBI and submitted with 3 local sequences to Mega X 10.2.4 software to obtain the Figure 12. Eleven global taxa about *blaTEM* gene of *P. aeruginosa* were downloaded from NCBI and submitted with 3 local sequences to Mega X 10.2.4 software to obtain Figure 13.

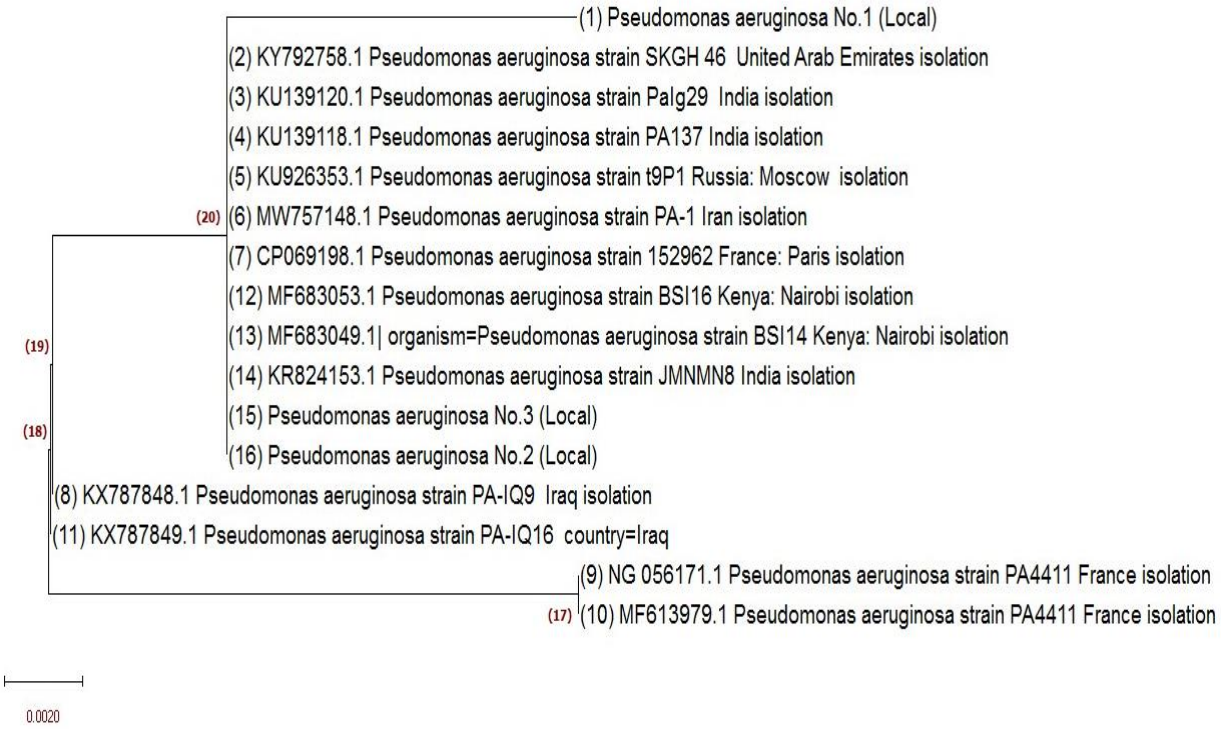

Figure 11: Phylogenic tree of blaCTX-M gene partial sequences of local and global sequences using neighbor joining bootstrap 1000 tree figure. Evolutionary relationships of 16 taxa. PA1 to PA3 represent the local isolates.

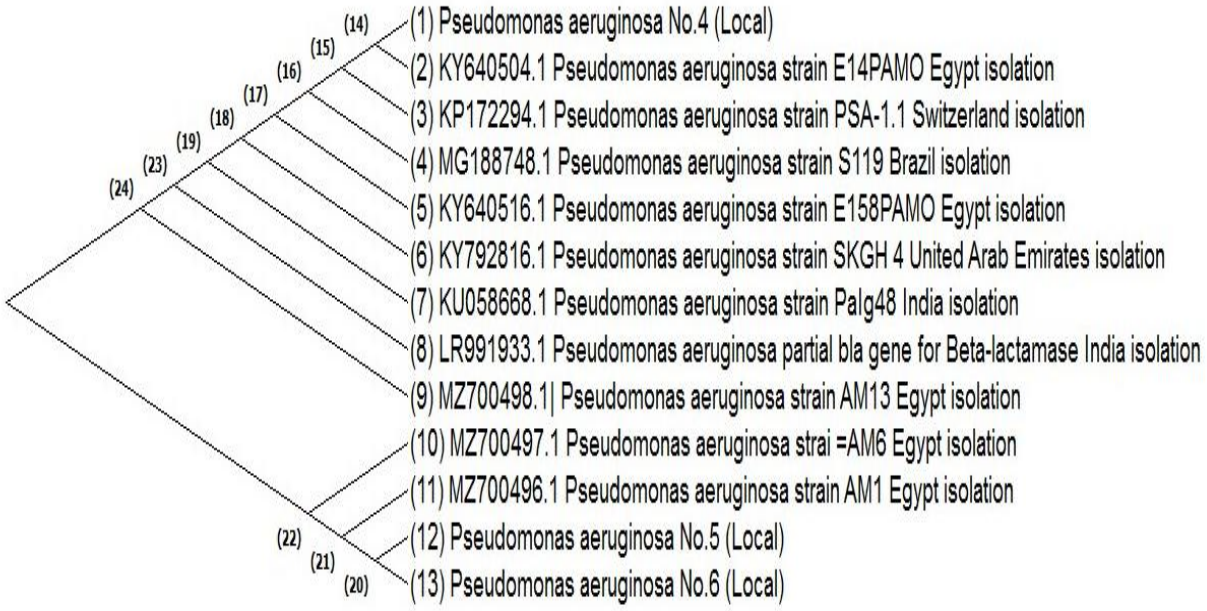

Figure 12. Phylogenic tree of blaSHV gene partial sequences of local and global sequences using neighbor joining bootstrap 1000 tree figure. Evolutionary relationships of 13 taxa. PA4 to PA6 represent local isolates.

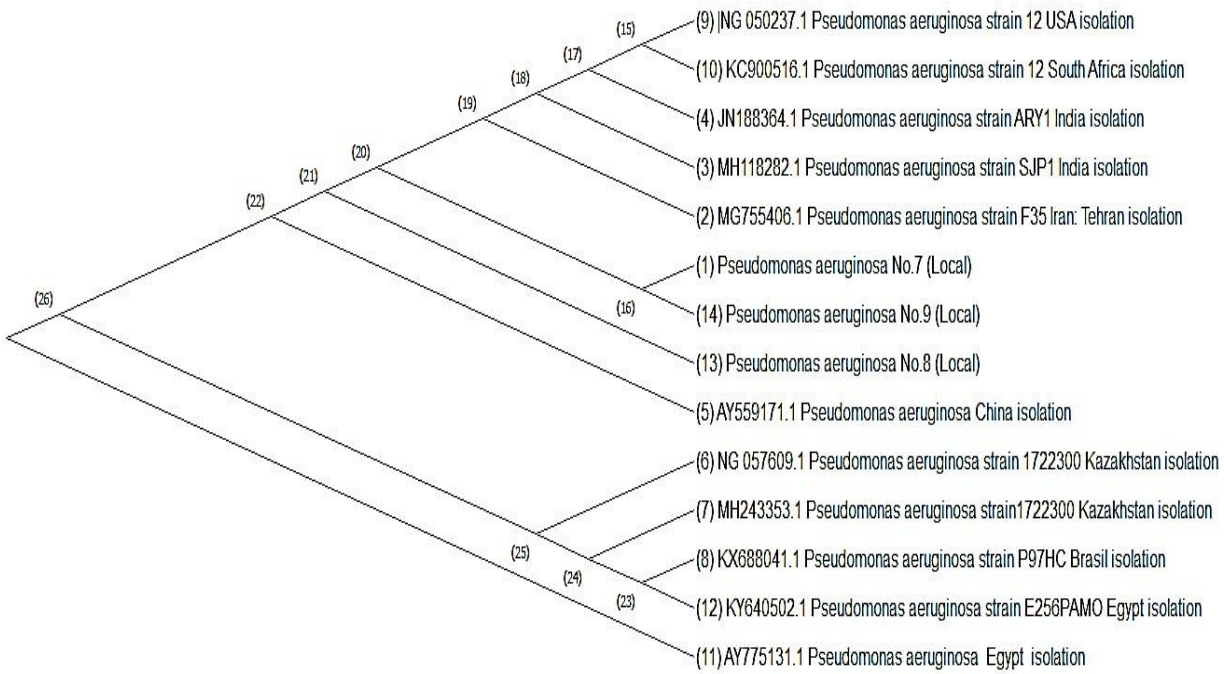

Figure 13: Phylogenic tree of blaTEM gene partial sequences of local and global sequences using neighbor joining bootstrap 1000 tree figure. Evolutionary relationships of 14 taxa.PA7 to PA9 represent local isolates.

MEGA features a number of useful tools for assembling sequence data sets from files or web-based repositories, as well as tools for visualizing the results in the form of interactive phylogenetic trees and evolutionary distance matrices [15]. The first stage in the analysis was to align all of the sequences form three genes in this study with other worldwide references using MEGA X 10.2.4 's (Clustal W) program step. This program was shown to have a high degree of similarity with all world sequences, including the sequences used in this study. These (Clustal W) results were significant since they were directly utilized in the phylogenetic tree design. The Neighbor-Joining (NJ) approach, which is a simplified version of the minimal evolution (ME) method, is used in this study to determine the close relationship between world and local sequences. Because it does not need the assumption of a constant rate of evolution, the NJ method yields an unrooted tree. An outgroup taxon is needed to find the root [10]. In bla*CTX-M* gene phylogeny (Figure 1), it was submitted 16 sequences, 3 sequences belong to local sequences and 13 sequences belong to global sequences obtained by download from NCBI they submitted to a MEGA X 10.2.4 software program for obtaining phylogenic relationship among local and global sequences, after submitting these sequences to MEGA X 10.2.4 at the first time we found alignment by Clustal W, then use NJ method at bootstrap 1000, the local sequence of *P. aeruginosa* PA1 was near to the sequence KY792758.1 *P. aeruginosa* strain SKGH (UAE) and Indian isolates (KU130118.1 and KU130120.1), while the local sequence of *P. aeruginosa* PA2 and PA3 was closely related to the sequence KR824153 .1 (Indian isolates). All three local sequences are far away from Iraqi isolates (KX787848.1 and KX787849.1). In bla*SHV* gene phylogeny as shown in Figure 12, It was found that both local sequences *P. aeruginosa* PA5 and PA6 were closely related to the sequences of Egyptian isolates (MZ700496.1 and MZ700497.1). Also, the local sequences of *P. aeruginosa* PA4 were closely associated with the sequence KY640504.1 strain E14PAMO which is isolated in Egypt (Table 6). In the phylogeny of *bla*$_{TEM}$ gene (Figer13), the local sequences *P. aeruginosa* PA7 and PA9 were closely related to each other to form sister sequences, related to sequence MG755406.1 *P. aeruginosa* strain F35 which isolate in Iran. In contrast, the local sequences *P. aeruginosa* PA8 are near to the sequence AY559171.1 which is isolated in China. Thus, phylogenic relationship among local and world strains provide high information about origin and genetic evolution of local isolates**.**

4. Conclusion

This study demonstrated significant variation in the virulence factors of Pseudomonas aeruginosa across geographic regions, including Iraq, UAE, India, Egypt, and Iran. Most isolates exhibited biofilm-forming ability, contributing to their antibiotic resistance and persistence in infections. The sequencing of blaTEM, blaCTX-M, and blaSHV genes revealed diverse genetic profiles, highlighting the need for further studies to create a comprehensive genetic diversity map of P. aeruginosa. The findings stress the importance of regulating antibiotic use to prevent the spread of resistance. Region-specific strategies and improved infection control measures are essential to combat resistant strains and ensure public health safety.

Declaration of Competing Interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary Materials:

No Supplementary Materials.

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The authors declare no conflict of interest.

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