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GENETIC VARIATION OF HOUSEKEEPING GENES IN MULTIDRUG RESISTANT *PSEUDOMONAS AERUGINOSA*

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Abstract: Background: Pseudomonas aeruginosa is a prominent opportunistic pathogen responsible for nosocomial infections, particularly among immunocompromised individuals. **Specific Background:** Its ability to develop multiple antibiotic resistance poses a significant clinical challenge, highlighting the need for a deeper understanding of its genetic diversity and virulence factors. **Knowledge Gap:** While previous studies have explored antibiotic resistance mechanisms, there is limited research on the genetic diversity of Pseudomonas aeruginosa isolates in specific geographic regions, such as Kirkuk. **Aims:** This study aimed to investigate the genetic diversity of Pseudomonas aeruginosa isolates from clinical samples obtained from Kirkuk Civil Hospitals, utilizing Multi-Locus Sequence Typing (MLST) for genetic analysis. **Results:** Fourteen P. aeruginosa isolates were confirmed through biochemical tests and the VITEK-2 system, with an alarming 85.71% (12/14) exhibiting antibiotic resistance. Molecular analysis revealed the presence of several housekeeping genes, although some genes did not amplify. Notably, two new serotypes (PS3:id:9797 and PS4:id:9796) were identified and added to the MLST database, along with three new genes registered in NCBI. Phylogenetic analysis indicated a divergent cluster among three isolates. **Novelty:** This research contributes new insights into the genetic diversity of Pseudomonas aeruginosa, identifying novel serotypes and genes, which are critical for understanding its epidemiology and resistance mechanisms. **Implications:** The findings underscore the importance of ongoing surveillance of Pseudomonas aeruginosa in clinical settings to inform treatment strategies and public health policies aimed at managing antibiotic resistance and improving patient outcomes.

Keywords: *Pseudomonas aeruginosa*, MDR. HKG, GD

Introduction

Pseudomonas aeruginosa is known as an opportunistic pathogen with a range of virulence(27). factors in humans including biofilms formation, toxins that causing broadtissue damage hence, accessto bloodstream and spreading to body tissue, as a role of colonization, invasion and persistence in human host), P. aerugenosa represents a phenomenon of resistance to many antibiotics creatinghard to treatinfections. This bacteria has natural resistance such as chromosomal expression, changing outer membrane permeability (2).In order to evaluate the genetic variations employed in

epidemiological research, it is necessary to determine the genetic relatedness between pathogenic isolates by phylogenetic and population-based analysis(26). Multilocus sequence typing (MLST) is a genotyping method that involves analyzing the nucleotide sequences and allelic differences of seven house-keeping genes (*acsA, aroE, guaA, mutL, nuoD, ppsA, and trpE*). It is commonly used to study the genetic diversity and investigate the epidemiology of *P. aeruginosa* (2). Infections resulting from the presence of multidrug-resistant (MDR) *P. aeruginosa* are correlated with substantial morbidity and death. The considerable presence of innate and acquired resistance to several antibiotics demonstrated by *P. aeruginosa* poses significant difficulties in its treatment and imposes limitations on available therapeutic strategies. *Pseudomonas aeruginosa* demonstrates a wide array of resistance mechanisms, encompassing nearly all currently recognized mechanisms. food-producing animals. *P. aeruginosa* belongs to the ESKAPE group (4). The assessment of the frequency of MDR bacteria, is of paramount importance in mitigating the potential hazards associated with illnesses caused by multidrug resistance. *Pseudomonas* possesses many virulence factors that help it attach to and invade various tissues in the body, causing many diseases by releasing several extracellular toxin and enzymes, ultimately leading to the formation of a biofilm (5). Multilocus sequence typing (MLST) is an alternative method for molecular typing which is a global and accurate strain-typing system that concentrates entirely on conserved housekeeping genes and the combination of each allele)7) and firstly identified by Curran and his group (8) advanced for *P. aeruginosa* by Curran *et al.*, (8) The MLST scheme is a method used to differentiate *P. aeruginosa* isolates based on variations in the sequences of seven specific genes: *acsA, aroE, guaA, mutL, nuoD, ppsA,* and *trpE*. This scheme offers a comprehensive database that facilitates the comparison of findings acquired from different locations and sample types and had the most analytical value (100%) in labeling strains as unique (9). The standardization of MLST has given increase to databases which allow comparative analysis of allele sequences and documentation of distinctive sequence types (7). The aim of the present study was to examine the genetic composition of *P. aeruginosa* isolates collected from clinical samples and to detecting the existence of virulence determining the sequence type (ST) for each isolate, and evaluating the similarity of the isolates at the core genome level.

Methods

Collection of specimens

The present study was conducted in Kirkuk city, during the period from January to August 2023.A total of 14 strains of *P. aeruginosa* were collected from clinical sources. Most of these clinical isolates which were obtained from hospitalized patients (both sexes and from different ages) who were admitted to both Azadi Teaching Hospital and Kirkuk Teaching Hospital. Informed consent was obtained from all participants.

Bacterial isolation and Identification

The collected samples were cultivated on various agar media including Blood agar, MacConkey agar, and Cetrimide agar. The inoculated plates were incubated at 37 C for 24 hr. The isolated bacteria were identified based on microscopic and cultural characteristics such as colony morphology, shape, size, color, odor, and pigment production and confirmatory tests using VITEK-2. Finally, the bacterial isolates were kept at 20C in Brain heart infusion broth supplemented with 15% glycerol.

Phenotypic and antibiotic susceptibility characterizations

Phenotypical characterization of fourteen isolates was conducted using the automatized VITEK®2 GN technique (bioMériux, France). Their antibiogram profiles were determined using

VITEK-2 in accordance with CLSI guidelines for all antibiotics. These isolates were categorized based on their resistance pattern into different groups: multidrug resistant (MDR), extensively drug resistant (XDR), pan drug resistant (PDR), and non-multidrug resistant (non-MDR). This classification was based on the fact that MDR isolates were non-susceptible to at least one agent in three or more antimicrobial categories(10).

DNA extraction and PCR amplification

For extraction of DNA The Wizard® Genomic DNA extraction Kit for Gram-negative bacteria (Promega, USA), was employed to isolate and purify DNA by following manufacturer instructions.Specific primers were used to amplify and sequence the housekeeping genes *acsA, aroE, guaA, mutL, nuoD, ppsA,* and *trpE* for the 14 isolates subsequently, as seen in Table (1), the collected DNAs were subjected to electrophoresis using a 1% agarose gel stained with ethidium bromide. The resulting gel was then seen under a UV transilluminator. The selected primers were taken from MLST based on the standard gene bank sequence provided by the National Center for Biotechnology Information (NCBI). The master mix used for gene amplification consisted of all the necessary components(8), namely the GoTaq® G2 Green Master Mix (Promega, USA). The PCR amplifications included of 35 cycles, with each cycle consisting of a denaturation step at 95°C for 30 seconds, annealing of different temperature ranged between 53°C, 55°C, 56°C, and 60°C for 30 seconds for each respective gene. Subsequently, an extension step was performed at 72°C for 50 seconds. These steps were carried out in a thermal cycler, with minor modifications made for optimization purposes.

Table 1: Housekeeping gene specific primers

Primer *		**Sequence**	**Tm (°C)**	**GC (%)**	**Product size**
acsA	Forward	5'- ACCTGGTGTACGCCTCGCTGAC-3'	68.3	70	842 base pair
	Reverse	5'GACATAGATGCCCTGCCCCTTGAT-3'	66.1	36	
aroE	Forward	5'- TGGGGCTATGACTGGAAACC -3'	63.1	45.8	1053 base pair
	Reverse	5'-TAACCCGGTTTTGTGATTCCTACA-3'	60.8	36.4	
guaA	Forward	5'- CGGCCTCGACGTGTGGATGA - 3'	67.8	70	940 base pair
	Reverse	5'GAACGCCTGGCTGGTCTTGTGGTA-3'	68.3	36	
mutL	Forward	5'- CCAGATCGCCGCCGGTGAGGTG - 3'	73.5	45.8	940 base pair
	Reverse	5'- CAGGGTGCCATAGAGGAAGTC -3'	63.2	36.4	
nuoD	Forward	5'- ACCGCCACCCGTACTG - 3'	63.6	70	1050 base pair
	Reverse	5'- TCTCGCCCATCTTGACCA - 3'	62	36	

ppsA	Forward	5'- GGTCGCTCGGTCAAGGTAGTGG - 3'	67.8	45.8	989 base pair
	Reverse	5'- GGGTTCTCTTCTTCCGGCTCGTAG-3'	66.9	36.4	
trpE	Forward	5'- GCGGCCCAGGGTCGTGAG - 3'	71.3	70	811 base pair
	Reverse	5'- CCCGGCGCTTGTTGATGGTT - 3'	66.1	36	

* Primers references *Pseudomonas aeruginosa* MLST database.

Allelic analysis and genetic diversity

The housekeeping genes *acsA, aroE, guaA, mutL, nuoD, ppsA,* and *trpE* have been chosen based on the MLST scheme for *P. aeruginosa* (http://pubmlst.org/paeruginosa/) as housekeeping genes. The MLST procedure was conducted following the methodology outlined by Curran *et al* (8) with minor modifications. The amplification reaction combination was used to amplify the housekeeping genes. The reaction conditions consisted of denaturation at 94°C for 1 minute, annealing at 58-60°C for 1 minute, and extension at 72°C for 1 minute. This process was repeated for 35 cycles, with a final extension at 72°C for 5 minutes. The PCR products were purified using the Mini Elute PCR Purification Kit (Promega, USA), following the manufacturer's instructions. The sequencing procedure utilized internal nested primers, as detailed on the http://pubmlst.org/P.aeruginos/info/primers.shtml website. The PCR results were subjected to purification before being sequenced. The forward and reverse sequences were imported into Geneious, version R8.1(11), where they were assembled, edited, trimmed, and confirmed. The final sequences were stored in Fasta format. Positions containing gaps in any of the aligned sequences were eliminated from the study. Each sequence was verified by several BLAST searches to ensure that the associated data aligned accurately with the relevant gene of *P. aeruginosa* (2). Confidence intervals for bootstrap probabilities based on 1000 replicates (2).

Locus selection for MLST analysis

The assignment of ST and alleles was conducted using *P. aeruginosa* MLST website (http://pubmlst.org/paeruginosa/). Sequences did not correspond to an established locus in the database were classified as "new" alleles. Furthermore, the novel allele combinations in the database that did not correspond to any of the new STs were also designated as "new". The present investigation involved determination of nucleotide sequences for each allele of each locus. New sequences were subsequently submitted to curator Eleanor Pinnock for inclusion in the *P. aeruginosa* MLST website (http://pubmlst.org/paeruginosa/).

Results and Discussion

Description of bacterial isolates

In the present study, the bacteriological results revealed that a total of 14 isolates of *P. aeruginosa*, The diagnosis was confirmed using cultural, microscopic, and biochemical examinations, and further validated with VITEK-2 as shown in Figure (1). These results were consistent with what has been reported by researchers. according to their sources. All *P. aeruginosa* isolates that were identified by selective agar culture media, biochemical and microscopic tests, were confirmed at the genus and species the diagnostic confirmation was done using VITEK-2.

Cetrimide agar has been seen to exhibit both pyocyanin and pyoverdine as characteristic colors

produced by *Pseudomonas aeruginosa*. The findings of this study indicate that almost 29% of the isolates had a distinct blue-green hue, a trait that can be related to the existence of the pyocyanin pigment. The presence of pyoverdine pigment, namely in the green-yellow color, was observed in 50% of the isolates. Furthermore, it was observed that 21% of the isolates produced a yellow-fluorescent pigment, as seen in Figures 2 and 3. However, the data presented above contradicts the results reported by Hameed *et al*. 2017, who found that only 31.03% of *P. aeruginosa* isolates possess the capability to generate pyoverdine pigment (15). The discrepancies identified in our results in relation to previous research can be attributed to variances in environmental circumstances throughout the differences in the specific strain of the isolates. Various variables have the potential to impact iron metabolism, resulting in changes in both iron intake and siderophore synthesis. These variances can exhibit significant disparities among different strains.

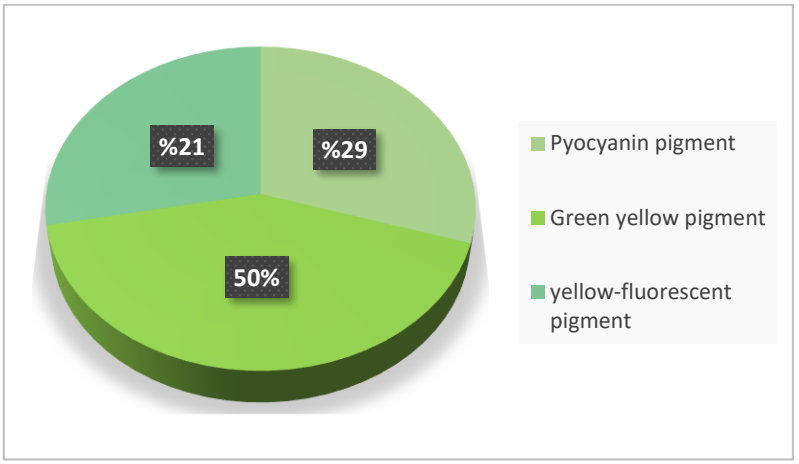


Figure (2) Pigments produced by *P. aeruginosa* on cetrimide agar**.**

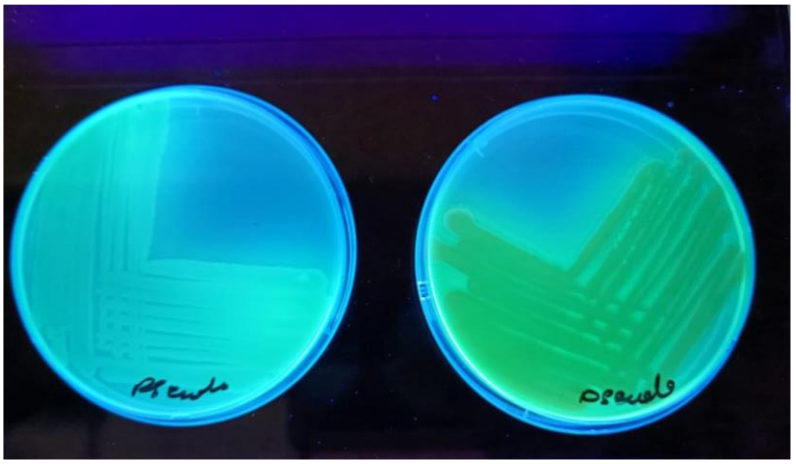


Figure (3): *P. aeruginosa on* Cetrimide agar showing fluorescent dye under UV. light.

Antibiotic resistance pattern

The results of the antibiotic sensitivity tests conducted on On the 14 P. aeruginosa isolates by using VITEK II system are displayed in Table 3. The antibiotics used in the study were ticarcillin, ticarcillin/clavulanic acid, piperacillin/tazobactam, levofloxacin, ciprofloxacin, ceftazidime, cefepime, imipenem, meropenem, amikacin, and gentamicin. The findings revealed diverse degrees of antibiotic resistance, with ticarcillin exhibiting the greatest percentage of resistant bacteria (96%), followed by ticarcillin/clavulanic acid (91%). The resistance rate of levofloxacin was shown to be 33%, whilst ciprofloxacin and ceftazidime had resistance rates of 30% and 39%. respectively The rates of resistance for cefepime and gentamycin were found to be 26%, whilst amikacin had a resistance rate of 30%. The results also revealed a high prevalence of Ticarcillin resistance, with a

rate of 96%, Table (3). On the other hand, imipenem and meropenem showed a sensitivity rate of 100%.

In the current study as shown in table (4), the antibiotic susceptibility profile classified these isolates as multidrug resistant (85.71%) and non-multidrug resistant (14.28%), with *P*. value (0.021) This present study aligns with the previous research conducted by Al-Shwaikh et al., 2018, which reported a resistance rate of 87% among the isolates (16). The term agent-resistant microorganism" pertains to the ability of a microorganism to exhibit resistance against one or more antibiotics that are especially designed to combat *P. aeruginosa* (6). Research conducted in Iran revealed that the antimicrobial agents with the highest levels of resistance were gentamicin, tobramycin, and cefoxitin, with a resistance rate of 100%. Conversely, all isolates exhibited susceptibility to colistin. A total of 14 isolates profiles were identified, with profile 1 exhibiting the strongest resistance in more than 50% of the isolates . However, our data are in line with Corehtash et al findings, who reported a notably high prevalence of multidrug-resistant (MDR) isolates in Iran, 93.1% of isolates were MDR, linking this phenomenon to extended hospital stays and inappropriate antibiotic usage (19) . In addition, a research done by Atilla et al., 2012 shown that a considerable percentage of individuals (77.3%) diagnosed with *P. aeruginosa* infection had resistance to Ticarcillin. The efficiency of Gentamicin against antibiotic-resistant *Pseudomonas aeruginosa* isolates was investigated in a study conducted by AL-Mayyahi et al., 2018, the findings of the study indicate that around 47.6% of the isolates exhibited resistance to Gentamicin (18). which can significantely enhance the ability of the microorganism to develop resistance to new generations of antibiotics. Moreover, *Heidari et al* (20), found that 60% of the isolates were multidrug-resistant (MDR). The findings of this investigation are in opposition to the conclusions drawn in a previous publication, which reported that 25.4% of multi-drug resistant (MDR) isolates of the bacterium shown resistance to Gentamycin (21). The observed disagreement between the findings of this study and those of previous publications may suggest the potential establishment of resistance patterns in the microorganism. The resistance seen may be ascribed to the improper utilization of antibiotics without seeking guidance from a healthcare practitioner or prematurely terminating the antibacterial therapy purely based on patient contentment. The prevention of future development and spread of multidrug-resistant strains requires strict attention to the instructions and suggestions supplied by the healthcare sector about the administration of antibacterials.

Table (3): Antibiotic sensitivity of isolated bacteria.

No.	TC	TIM	LEV	CIP	CAZ	CPM	IMI	MER	AK	CN	MDR
P1	R	R	S	R	R	S	R	S	S	S	50%
P2	R	R	R	R	R	S	R	S	S	S	60%
P3	R	R	S	R	R	R	R	S	S	S	60%
P4	R	R	R	R	R	R	R	S	S	S	70%
P5	R	R	R	R	R	S	R	S	R	R	80%
P6	R	R	R	R	R	R	R	R	R	R	100%

P7	R	R	S	R	R	R	R	R	S	S	70%
P8	R	R	R	R	R	S	R	S	S	S	60%
P9	R	R	R	R	R	R	R	S	R	R	90%
P10	R	R	S	R	R	S	R	S	S	S	50%
P11	R	R	S	R	R	R	R	R	R	R	90%
P12	R	R	R	R	R	R	R	R	S	S	80%
P13	R	R	R	R	R	R	R	S	R	R	90%
P14	R	R	S	R	R	R	R	S	S	S	60%

Table (4): The percentages of MDR and antibiotics resistance *P. aeruginosa*

Multi Drug Resistant	***P. aeruginosa***		***P***
	No.	**%**	**value**
Multi Drug Resist (MDR)	**12**	**85.71429**	**0.021**
Non-multi drug resistance	**2**	**14.28571**	
Total	**14**	**100**	

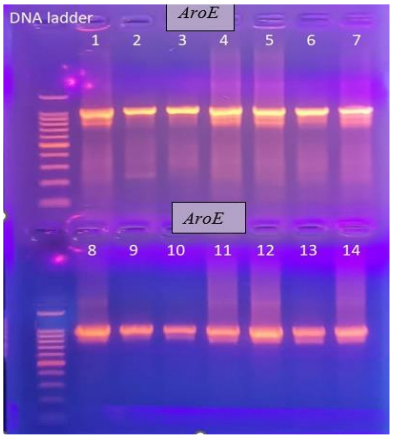
Genomic DNA extraction and PCR amplification of housekeeping genes in P. aeruginosa

DNA extraction and amplification of 7 housekeeping genes (aroE, mutl, acsA, trpE, nuoD, guaA, PPSA) were successfully performed as illustrated in Figure 4. Allele profiles and STs can be found at (http:// pubmlst.org/paeruginosa). By comparing the sequence and combination of genes the clonal relationship between isolates can be assessed (8). It has been reported that isolates with the same sequence types (ST) can be considered as members of the same clone, and P. aeruginosa isolates that share at least five of the seven numbers within their allelic profile were regarded as members of the same clonal complex (7). resulting in a product size of 1053 base pairs. Subsequently, the amplified samples were subjected to agarose gel electrophoresis. The gel electrophoresis bands were arranged in the following sequence: as seen in Figure 4, The presence of the *trepE* gene was observed in 78.57% of the isolates. The *nuoD* gene was detected in 85.71% of the isolates, *guaA* gene was observed in 71.42% of isolates, finally *PpsA* was detected in 100% of isolates. Some genes could not be amplified due to various reasons, such as DNA concentration, PCR conditions, or because the primers might not be specific for all bacterial isolates.

Table (5): Distribution of housekeeping genes of *P. aeruginosa*

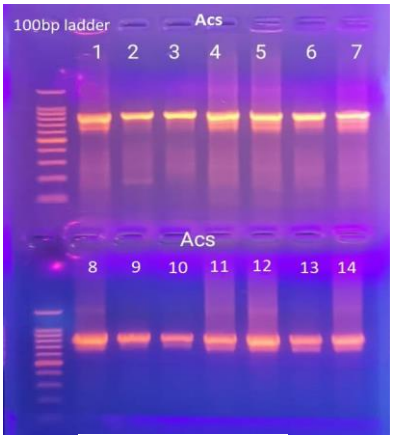
House keeping gene	**Positive**		**Negative**		**P. value**
Total = 14	**No.**	**%**	**No.**	**%**	
***aroE* gene**	**14**	**100**	**0**	**0**	**0.0031**
***acsA* gene**	**14**	**100**	**0**	**0**	

***trpE* gene**	**11**	**78.57143**	**3**	**21.4286**
***nuoD* gene**	**12**	**85.71429**	**2**	**14.2857**
***guaA* gene**	**10**	**71.42857**	**4**	**28.5714**
***PPSA* gene**	**14**	**100**	**0**	**0**
MutL gene	**14**	**100**	**0**	**0**



A

B



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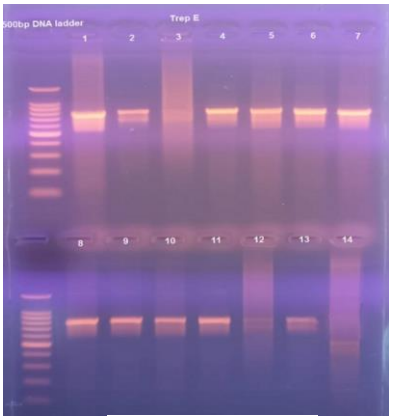

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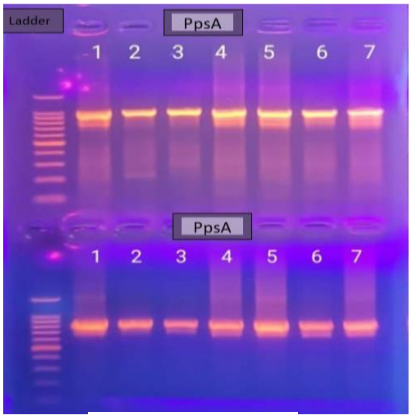

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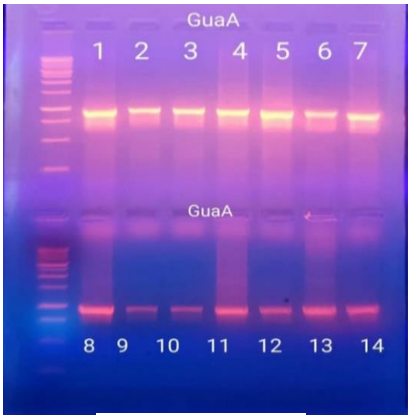

F

G

Figure (4): Gel electrophoresis reveals PCR product of *aroE, mutL, acsA, trpE, ppsA, guaA* and *nuoD,* gene as (A, B, C, D, E, F, G) respectively. The products were run in 1% agarose gel at 5 volt/cm^{2}for 1hr. The first Llane on the left corresponded to a ladder, L lane represents DNA ladder. The lanes 1-14 indicating amplified genes.

The cause of the variation in isolated *P. aeruginosa* from clinical samples is not understood; it might be due to true disparities between *P. aeruginosa* populations found in various geographical locations. However, it is important to exercise caution as PCR-based analysis might produce false negatives. The existence of the gene in *P. aeruginosa* is a changeable feature that is evident (22).

The findings of the present results suggest that individuals in this location who are infected with *P. aeruginosa* are at a significant risk of developing antibiotic resistance. The increased resistance rate can be attributed to previous exposure to several antibiotics and the use of empirical combination treatment. The excessive use of antibiotics, the propagation of resistant strains, the introduction of low-quality or non-WHO prequalified drugs into the market, and self-medication are likely the primary causes of the high incidence of antibiotic resistance (2). The findings in the current study are consistent with a previous investigation conducted by (Al-Tememe and Abbas, 2022) on the molecular amplification of the *aroE* gene. The study revealed that out of the 42 bacterial isolates examined, all provided a positive result. Additionally, the size of the amplified gene was around 495 base pairs. According to another study, a total of six clinical isolates of *P. aeruginosa* were identified through in-silico MLST analysis. These six strains, namely PA_64, PA_65, PA_88, PA_107, PA_141, and PA_152, exhibited distinct allelic profiles for seven housekeeping genes (*acsA, aroE, guaA, mutL, nuoD, ppsA,* and *trpE*), which are commonly employed for multi-locus sequence typing of *P. aeruginosa* strains. The pan-genome is defined as the collective genetic information contained inside a group of bacterial genomes. The concatenation of the housekeeping genes from each strain was performed by research conducted by (Aguilar-Rodea *et al*., 2022) determined that *P. aeruginosa* exhibits a significant level of genetic recombination and population diversity. Nonetheless, despite this diversity.

Allele profile and genetic variation of house keeping genes

The allelic and nucleotide diversities were calculated from the gene sequences using the Bioedite software to detect any variation in the House keeping genes All allele sequences were discovered in the database, and none of the seven genes had novel alleles. For each isolate, the combination of alleles obtained at each locus defined its allelic profile Individual phylogenetic trees and concatenated analyses of the sequenced gene fragments were constructed as seen in Table (6). or sequence type (ST). Polymorphic sites are seen, together with low amounts of nucleotide diversity per site .Furthermore, if a strain exhibited a novel combination of alleles, with at least one allele differing among the seven genes analyzed, it would be categorized as a new sequence type. The allele combinations of all newly discovered sequence types were submitted and deposited in the *P. aeruginosa* MLST database, which can be accessed at (http://pubmlst.org/paeruginosa), along with their respective sequence types (ST). The genetic profiles for each analyzed gene and their matching sequence type (ST) numbers are provided in Table 7. The first study in Kirkuk recorded only two isolates of Pseudomonas aeruginosa in MLST, bringing the total number of registered isolates in Iraq to 24. Four isolates were selected for genetic analysis and phylogenetic tree construction. The genetic sequences of the seven genes of the four Pseudomonas aeruginosa isolates were compared. Two new isolates, PS3 and PS4, were registered in MLST under the names of the student and supervisors. After comparing our results with those of other studies, the findings showed the spread of different types

of Pseudomonas aeruginosa, reflecting genetic diversity. The results showed that three isolates clustered together, indicating genetic proximity among the three isolates, while the fourth isolate showed genetic divergence from the rest, indicating genetic diversity. These isolates were collected from the same hospital. The results also showed genetic divergence between the isolates under study and the isolates registered in other provinces (Erbil, Duhok, Sulaymaniyah, Tikrit), indicating that Pseudomonas aeruginosa has the ability for genetic variation and adaptation to environmental conditions.. Various varieties exist in Iraq.

The identification of these sequence types (STs) that displayed extensively drug-resistant (XDR) and highly virulent characteristics within our healthcare facilities undoubtedly represents one of the most formidable pathogenic strains of Pseudomonas aeruginosa. However, 10 isolates, all of which were designated as novel sequence types (STs), were not grouped with any of these clusters and were determined to be unrelated, exhibiting minimal or no shared alleles. This implies that each of these novel sequence types (STs) in Kurdistan may have originated from different evolutionary sources(2).

This finding suggests that there is a combination of closely related isolates that share a recent common ancestor, as well as some more distantly related strains, within the examined strain collection. In addition, the Mantel tests yielded statistically significant relationships. The results indicate that there is no significant association between the genetic background of these isolates and the precise locations of infection.

Strains of *P. aeruginosa* obtained from various geographical locations and infection sites exhibited differences in their allelic profiles and STs, which might indicate their capacity to adapt to varied environmental habitats and the range of infections and disorders they cause. The predominance of *P. aeruginosa* in this location poses a significant danger of high antibiotic resistance. This is a high-risk clone of *P. aeruginosa*. Consequently, accurate identification and characterization of this bacteria are essential. Establishing the genetic relatedness among highly pathogenic strains of a virus in clinical settings is essential for long-term epidemiological research. This information can have beneficial implications for diagnosis, antimicrobial therapy, and infection control measures.

Table 7: Genetic diversity of the selected loci among the *Pseudomonas aeruginosa* isolates analyzed in this study

Locus	No of isolates	nucleotide length (bp)	No of alleles	Gene diversity ± SD	No of polymorphic sites	Avg. number of nucleotide differences	Nucleotide diversity ± SD
acsA	4	631	27	0.03125 ± 0.177	26	13.5	0.0215 ± 0.00444
aroE	2	502	2	0.25000 ± 0.5	2	3	0.00602 ± 0.00492
guaA	4	791	17	0.03125 ± 0.177	17	8.667	0.01111 ± 0.00288
mutL	2	379	1	1 ± 0.5	1	1	0.00264 ± 0.00264

nuoD	4	602	3	0.07031	±	3	1.5	0.00250	±
				0.265				0.00157	
ppsA	3	445	8	0.833	±	8	4.5	0.01020	±
				0.222				0.00350	
trpE	4	117	54	0.833	±	57	28.667	0.28383	±
				0.222				0.03969	

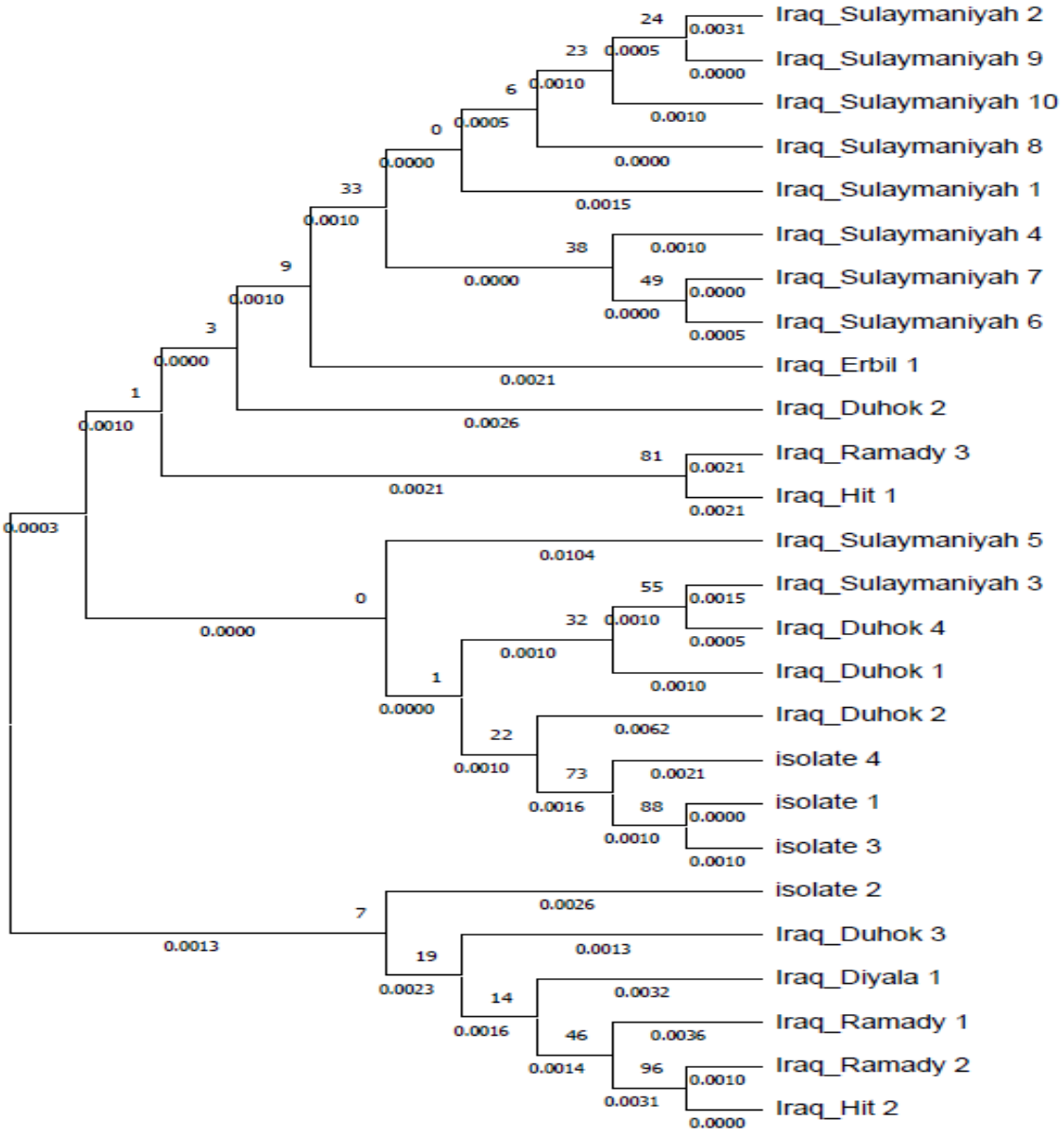

Figure 6: Concatenated phylogenetic tree showing evolutionary relationships of the seven genes analyzed (acsA, aroE, guaA, mutL, nuoD, ppsA and trpE) between the studied clinical *Pseudomonas aeruginosa* and other isolates

New bacterial strain identification:

Two new strains have been discovered and registered under the names of the student and supervisors. (PS3, PS4) were identified as global new Iraqi strain due the mutation in some nucleotide position. This new strain AS-85U was published in the MLST database https:// pubmlst.org/paeruginosa/with(PS3id:9797)(PS4:id9796).

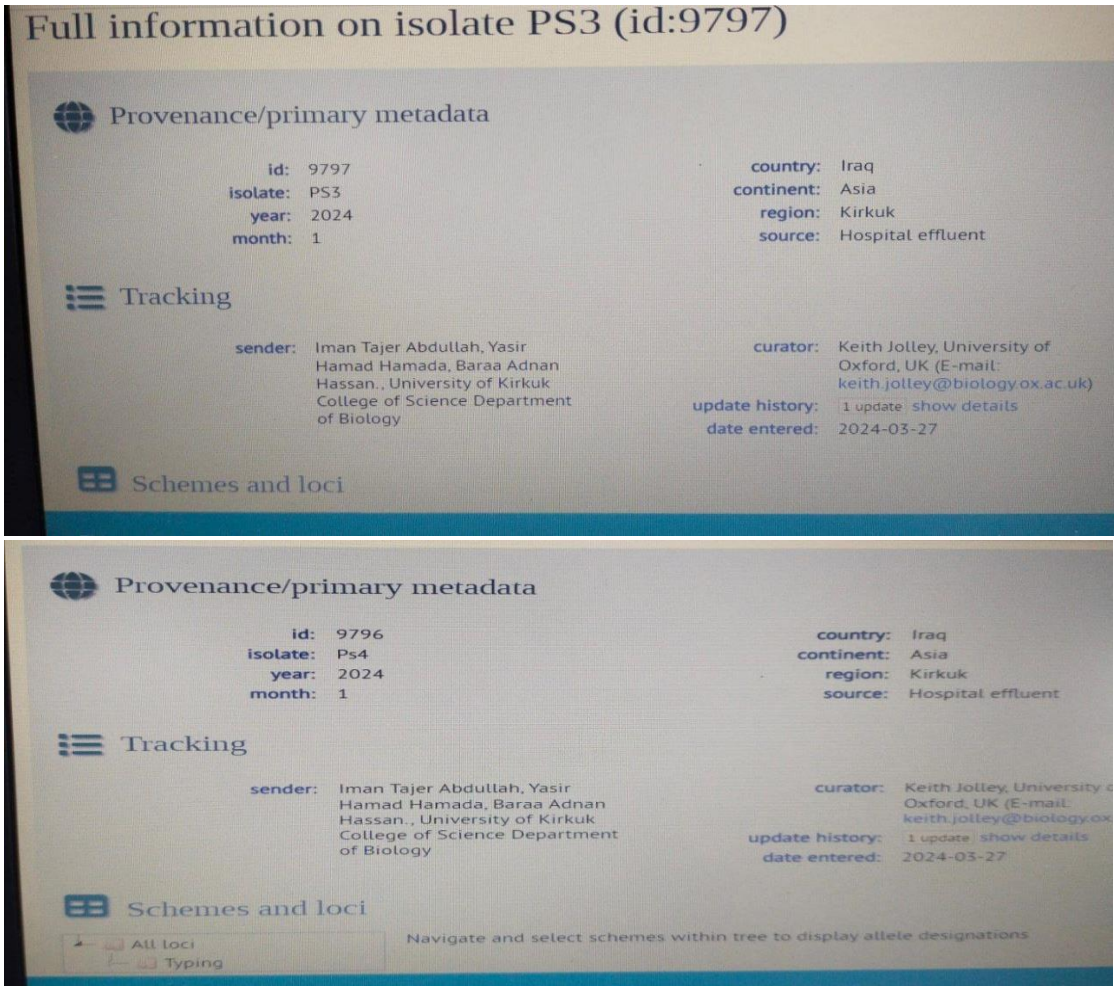

Figure 7: Bacterial isolates(id:9798, id:9796) registered in NCBI under the names of the student and supervisors

GenBank Send to:

Pseudomonas aeruginosa strain Burn_2 acetyl-coenzyme A synthetase gene, partial cds

GenBank: PP731982.1
FASTA Graphics

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  AUTHORS   Hamada,Y.H., Abdullah,I.T. and Hassan,B.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-APR-2024) University of Kirkuk, Kudus street, Kirkuk
            36001, Iraq
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GenBank

Pseudomonas aeruginosa strain 4 AcsA (acsA) gene, partial cds

GenBank: PP801284.1

FASTA Graphics

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  AUTHORS   Abdullah,I.T., Hamada,Y.H. and Hassan,B.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-MAY-2024) Abdullah, University of Kirkuk, university
            street, Kirkuk 36001, Iraq
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GenBank

Pseudomonas aeruginosa strain Burn1 anthranilate synthase component I (trpE) gene, partial cds

GenBank: PP780188.1

FASTA Graphics

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  ORGANISM  Pseudomonas aeruginosa
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REFERENCE   1  (bases 1 to 1368)
  AUTHORS   Hassan,B.A., Abdullah,I.T. and Hamada,Y.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-MAY-2024) Abdullah, University of Kirkuk, university
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Figure 8: Genges registered in the gene bank

Conclusion

Pseudomonas aeruginosa possesses multiple genetic components that contribute to its pathogenicity, including virulence and resistance to antibiotics. The study reveals a correlation

between the resistance of *P. aeruginosa* to most antibiotics, indicating a multidrug resistance to antibiotics. Molecular methodologies offer very sensitive analytical instruments for the purposes of identification, sequencing, and enhancing the quality of epidemiological inquiries. The recently established Multi-locus Sequence Typing (MLST) approach for *P. aeruginosa* recommends a precise diagnosis of the bacterium and identification of the origin of infections and therapy. New Iraqi bacterial strain has been identified and recorded in database of MLST (PubMLST) under the name (PS3, PS4).

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