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Molecular diagnosis of *Pseudomonas aeruginosa* isolated from clinical and milk samples

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Abstract. *Al-Mozan HDK. 2024. Molecular diagnosis of* Pseudomonas aeruginosa *isolated from clinical and milk samples. Biodiversitas 25: 2648-2655.* The extensive use of antiseptic and disinfectants, which are not free of quaternary ammonium compounds, which has occurred recently, led to the emergence of drug-resistant bacteria. The current study aimed to determine the accurate method of identification, the presence of *QacE*, and *QacEΔ1* genes in *Pseudomonas aeruginosa* isolated from milk compared to *P. aeruginosa* of clinical isolates, and the relationship between the presence of *QacE, QacEΔ1* genes, and multidrug resistance. Therefore, 27 clinical and 34 milk samples were collected and examined by biochemical and molecular methods to detect *P. aeruginosa* bacteria. Also, a susceptibility test was performed. The results exhibited that 13 (21.3%) isolates were identified as *P. aeruginosa* using biochemical methods, 8 (13.1%) isolates were identified as *P. aeruginosa* by molecular method; the *QacE* and *QacEΔ1* genes were 100% and 83.3% for clinical isolates and 50% and 100%, respectively, for milk isolates. Both isolates showed superior resistance to ceftazidime and vancomycin, but the clinical isolates outperformed the milk isolates by 50% to ciprofloxacin, and imipenem. Clinical isolates were resistant to a greater number of drugs compared to milk isolates. The isolates with the accession numbers (LC805650, LC805652, and LC805651) were registered as new strains. Expression of *QacE, QacEΔ1* genes should be examined to know the true relationship.

Keywords: Multi-drugs resistance, *Pseudomonas aeruginosa*, *QacE* gene, *QacEΔ1* gene, *16S rRNA* gene

INTRODUCTION

This *Pseudomonas aeruginosa* bacterium is motile and negative to Gram staining (Fujitani et al. 2017; Karami et al. 2019); it has the ability to survive anywhere (Shukla and Mishra 2015; Swetha et al. 2017; Qin et al. 2022), including inert materials. *Pseudomonas aeruginosa* is considered among the most representative species of the genus *Pseudomonas*, which causes spoilage of animal products (Shahat et al. 2019; Jawher and Hassan 2023); thus, it is the cause of serious illnesses. It is a bacterium known to cause nosocomial infections (Addis et al. 2021; Haeidari et al. 2021), occupying the fourth rank among nosocomial pathogens. This bacterium is not only an opportunistic bacterium (Azu and Onyeagba 2006), but it is the most well-known to cause nosocomial infections because it can live in all environments and it is resistant to antibiotics and disinfectants (Dubois et al. 2001). It is the cause of many severe infections (Migiyama et al. 2016; Tsao et al. 2018), such as skin inflammation, respiratory tract infections, soft tissue infections, digestive tract infections, bacteremia, bones and joints, and others (Swetha et al. 2017), especially in immunocompromised individuals (Migiyama et al. 2016; Tsao et al. 2018; Bhardwaj et al. 2020). Therefore, its presence is not just a concern but a significant threat (Swetha et al. 2017), where it can be isolated from clinical samples (Al-Bayati et al. 2021).

Milk is considered one of the means of transporting Gram-negative bacteria present in the environment to humans (Garedew et al. 2012), where milk is an animal product known to have high nutritional value for humans. Therefore, it is a refreshing medium for microbial growth. Microbial contamination guarantees the spread of food poisoning (Bashir et al. 2014). *Pseudomonas* is considered one of the most well-known psychrotrophic bacteria causing spoilage of milk and its products, which has been shown to have many properties that make it resistant to many antibiotics (Meng et al. 2020). The ability of *Pseudomonas* to multiply in cold temperatures is the qualification that made it the largest percentage of the bacterial species that contaminate milk (Von Neubeck et al. 2015).

The proteolytic enzymes of *Pseudomonas* bacteria make milk and its products a favorable environment for bacterial growth, allowing these bacteria to contaminate food as quickly as possible (Atia et al. 2022). *Pseudomonas aeruginosa* infection is a very important problem due to its resistance to many drugs (Diggle and Whiteley 2020), especially because drug-resistant *Pseudomonas* is the main cause of the spread of infectious diseases (Gomi et al. 2017). The increase of antibiotic resistance and its spread between pathogenic and commensal bacteria occurs due to strong selective pressure resulting from excessive or indiscriminate drug intake or adding those antibiotics to food (Decimo et al. 2016).

Excessive use of antiseptics and disinfectants containing quaternary ammonium compounds (*QACs*) stimulated the emergence of antibiotic-resistant bacteria (Boyce 2023). However, among the virulence genes related to resistance to antiseptic and disinfectants contained by *P. aeruginosa* are *QacE* (Quaternary ammonium compound) and *QacE*

Δ1 (Quaternary ammonium compound delta) genes, which are responsible for the occurrence of mutual resistance between the disinfectants used and antibiotics (Hassuna et al. 2015). Determinants of *Qac* gene are located with genes encoding multiple resistance to some antibiotics (Wang et al. 2007), and *QacEΔ1* gene acts as a multidrug transfer gene (Mahzounieh et al. 2014).

The current study aims to determine the best method to identify *P. aeruginosa* and determine the most dangerous isolates in terms of resistance to antibiotics and possessing of *QacE*, and *QacEΔ1*genes and the relationship between the presence of *QacE*, and *QacEΔ1* genes and bacterial resistance to more than three antibiotics.

MATERIALS AND METHODS

Collection of samples

Therefore, 27 clinical samples (16 sputum samples from patients with respiratory infections and 11 wound swabs from patients with wound infections) and 34 milk samples were collected and examined for the presence of *P. aeruginosa* bacteria, where clinical samples were collected from patients who visited Imam Hussein Teaching Hospital from all districts of Thi-Qar Province for obtaining on the treatment, and raw milk samples were collected from local markets in Nassiriyah City Center of Thi-Qar Province, Iraq during the period from September 2022 to April 2023.

Isolation and identification of bacteria

Morphological and biochemical methods

Clinical samples. The bacterium was isolated and identified as *P. aeruginosa* by streaking of sputum sample or wound swab on MacConkey agar and incubated at 37°C for 24 hours. A Gram stain (Al-Badri 2021) knows changing by determining the bacterium's shape. Pure colonies of the fresh culture of the tested bacterium were subjected to catalase test, oxidase test (Qazangi 2017), hemolysin test (Javaid and Rashid 2018), fermentation test (Qazangi 2017), and Indol, Methel red, Voges-Proskauer, Citrate utilization tests (Collee et al. 1996), as well as API 20E (Qazangi 2017) system was used to confirm the identification.

Milk samples. The bacterium was isolated from milk samples using the pour plate method (Al-Mozan 2023). When pure colonies were obtained, the bacterium was subjected to the abovementioned tests, which diagnose bacteria in clinical samples.

Molecular identification method

Extraction of DNA. The DNA extraction process was applied to the bacterial isolates diagnosed as *P. aeruginosa*. The extraction was done according to the procedure of the Presto Mini DNA Bacteria Kit (Geneaid company).

Identification of bacterial isolates by *16S rDNA* gene. The polymerase chain reaction program for amplifying the target *16S rDNA* gene was an initial denaturation of 96°C for 3 min, 27 cycles including 96°C for the 30s, annealing 56°C for 25s and elongation temperature at 72°C for 15 s and final elongation at 72°C for 10 min., where 1 µL of each one of universal primers which were F: AGAGTTTG ATCCTGGCTCAG and R: GGTTACCTTGTTACGACTT (Miyoshi et al. 2005) was mixed with1 µL of DNA template, 9.5 µL of nuclease-free water, and 12.5 µL of master mix (Alyousif 2021). The amplified PCR products of *16S rDNA* genes were sent to Macrogen company (South Korea) for purifying and sequencing.

Examination of *P. aeruginosa* resistance to some antibiotics

The disk diffusion method was used to examine the resistance of *P. aeruginosa* to some antibiotics (Qazangi 2017), Figure 1. Results were determined depending on the size of the inhibition zone provided by the Clinical and Laboratory Standards Institute (CLSI 2023).

Detection of virulence genes (*QacE* gene and *QacEΔ1* gene)

Pseudomonas aeruginosa resistance genes to antiseptic and disinfectants were examined using specific primers F: ATGACCAACTATCTCTACCT and R: AACAACTGGATCAC CAGCA for *QacE* gene with 311 bp size, and F: GAAAGGCTGGCTTTTTCTTG and R: GCAATTATGAGCCC ATACC for *QacE1Δ* gene with 285 bp size (Abdullah 2018). The cycles required for the amplification were only one for each stage, except the denaturation stage required 30 cycles. The conditions consisted of the initial denaturation stage, which was performed under 95°C temperature for 5 minutes; the denaturation stage was done at 95°C temperature for 30 seconds; the annealing stage was performed at 55°C for 30 seconds; the extension stage was performed at 72°C for 45 seconds; and final extension stage at 72°C for 5 minutes for amplifying *QacE* gene. The same conditions were used to amplify *QacEΔ1* gene, except the temperature of the annealing stage was 55.5°C.

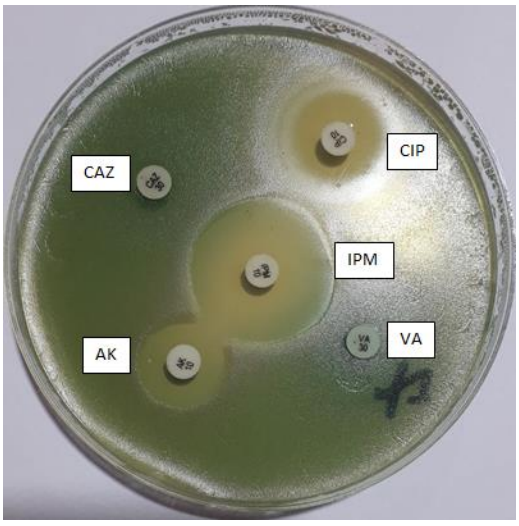

Figure 1. Susceptibility test of *Pseudomonas aeruginosa* to some antibiotics

Analysis of *16S rDNA* gene product sequence

The obtained *16S rDNA* gene product sequences for forward and reverse primers (13 isolates) were edited with the Bioedit program and compared with sequences of reference isolates deposited at NCBI using BLAST tools to identify the isolates.

RESULTS AND DISCUSSION

Identification methods of *P. aeruginosa*

Only eight isolates of *P. aeruginosa* were identified by examining 61 different samples (27 clinical samples and 34 milk samples) using biochemical and molecular tests. It should be mentioned that the biochemical test diagnosed five isolates with numbers (4,6,10,11,12) as *P. aeruginosa*, but the molecular test diagnosed these isolates as another bacteria, *Aeromonas* and *Proteus.* The biochemical method diagnosed *P. aeruginosa* with 37% in clinical samples and 8.8% in milk samples. At the same time, these bacteria were diagnosed with 22% in clinical samples and 5.9% in milk samples by molecular method Table 1. The isolates diagnosed with the molecular method as *P. aeruginosa* bacteria were recorded in GenBank with accession numbers shown in Table 2.

The molecular method was more accurate for diagnosing bacterial isolates than the biochemical methods, which is aligned with (Al-Hilali and Al-Mozan 2023). The molecular method based on diagnosing *16S rRNA* gene in bacteria and knowing its sequence is the most accurate because this gene contains variable regions that are different between bacterial species; therefore, knowing its sequence, the bacterial species is accurately determined. Also, this gene contains other constant regions unaffected by mutations, unlike biochemical methods that rely on the effectiveness of genes that may be disrupted due to emergency conditions. Thus, biochemical methods may fail to find the right bacterial species (Al-Mozan 2023).

An inaccurate diagnosis of non-pathogenic *Pseudomonas* species may lead to incorrect rulings about the suitability of food products (Dufour et al. 2008). Polymerase chain reaction technology is the best method for microbiology diagnosis (Shamkhi and Khudaier 2020).

Pseudomonas aeruginosa was found in clinical samples at 22%, which is inconsistent with (Al-Azzawi 2018), who recorded *P. aeruginosa* with rates of 68.6% and 69.2% in the wound and burn samples, respectively. It should be mentioned that *P. aeruginosa* was found in milk samples at 5.9%, which agrees with (Swetha et al. 2017), which recorded 15.2%, and disagreement with (Atia et al. 2022), with a percentage of 20%.

The difference in percentages may be due to the difference in samples included in the study, the difference in diagnosis methods, the difference in the regions from which the samples were collected, or the difference in the seasons during which the samples were collected.

Recording of new strains

The identification rate of the isolates was identified as *P. aeruginosa* in this current study when comparing their sequences with the sequences of other isolates stored at the National Center for Biochemical Information (NCBI) was 100% for isolate number 1 was isolated from wound swabs and recorded with accession number LC805648 strain HDKA1; wound isolate number 2 had accession number LC805649 strain HDKA2; isolate number 7 was isolated from sputum sample had accession number LC805653 strain HDKA7; isolate number 9 was isolated from wound swab where this isolate had accession number LC805646 strain HDKA9, and sputum isolate number 13 had accession number LC805647 strain HDKA13, except for three isolates with numbers 3, 5, and 8 which were recorded as new strains as shown in Table 2. Where A: A transition mutation was found when comparing of nucleotides sequence for isolate number 3, which was *P. aeruginosa* strain HDKA3 with (LC805650 accession number) isolated from milk in this study with reference isolate with (AB920753 accession number), where Adenine (A) at the position of 560 was replaced by Guanine (G)., B: a transversion mutation was found in this isolate that resulted in Thymine (T) at the position of 946 being replaced by Adenine (A) Figure 2.

Table 1. Comparison between biochemical test and molecular test to diagnose *Pseudomonas aeruginosa* in milk and clinical samples

Type of sample	Examined samples	Biochemical test	Molecular test
Clinical samples	27	10 (37%)	6 (22 %)
Milk samples	34	3 (8.8%)	2 (5.9%)
Total samples	61	13 (21.3%)	8 (13.1%)

Table 2. Accession numbers of isolates that were identified as *Pseudomonas aeruginosa*

No. of isolate	Source of isolate	Accession number of the current isolates	Strain	*16S rDNA* gene fragment size	Identity	Accession no. of reference isolates
1	Wound	LC805648	HDKA1	1378 bp	100%	MT337423
2	Wound	LC805649	HDKA2	1378 bp	100%	MT337423
3	Milk	LC805650	HDKA3	918 bp	99.45%	AB920753
5	Milk	LC805652	HDKA5	903 bp	99.59	AB920753
7	Sputum	LC805653	HDKA7	989 bp	100%	CP132949
8	Sputum	LC805651	HDKA8	907 bp	99.73%	AB920753
9	Wound	LC805646	HDKA9	1378 bp	100%	MT337423
13	Sputum	LC805647	HDKA13	1378 bp	100%	MT337423

As well as C: A transition mutation was found when comparing of nucleotides sequence for isolate number 5, which was *P. aeruginosa* strain HDKA5 with (LC805652 accession number) isolated from milk in this study with reference isolate with (AB920753 accession number), where Adenine (A) at the position of 559 was replaced by Guanine (G). In addition to D, a deletion mutation was found in this isolate in which Cytosine (C) at the position of 798 was deleted Figure 3.

However, E: A deletion mutation was found when comparing of nucleotides sequence for isolate number 8, which was *P. aeruginosa* strain HDKA8 with (LC805651 accession number) isolated from sputum in this study with reference isolate with (AB920753 accession number), where Guanine (G) at the position of 654 was deleted. Also, F: a transversion mutation was found in the mentioned isolate, where Guanine (G) at the position of 688 was replaced by Cytosine (C) Figure 4.

Mutation occurs in an organism either due to changing its place of living, exposure to environmental conditions, or chemical mutagens (Ilmjärv et al. 2017). It results in a change in DNA sequence (Qazangi and Jabr 2017) that cannot be repaired, and as a result, it becomes inherited (Ilmjärv et al. 2017).

Isolates have the same chance of being exposed to mutation, as two isolates from milk were among three isolates in this study in which mutations occurred and were recorded as new isolates. The reason may be that the isolate was transmitted from an infected person to milk, or due to sterilizing the udder with disinfectants, lack of hygiene during the milking process, or giving the animal antibiotics to increase growth. The same applies to the isolate from sputum, as the patient took antibiotics and used gargling solutions.

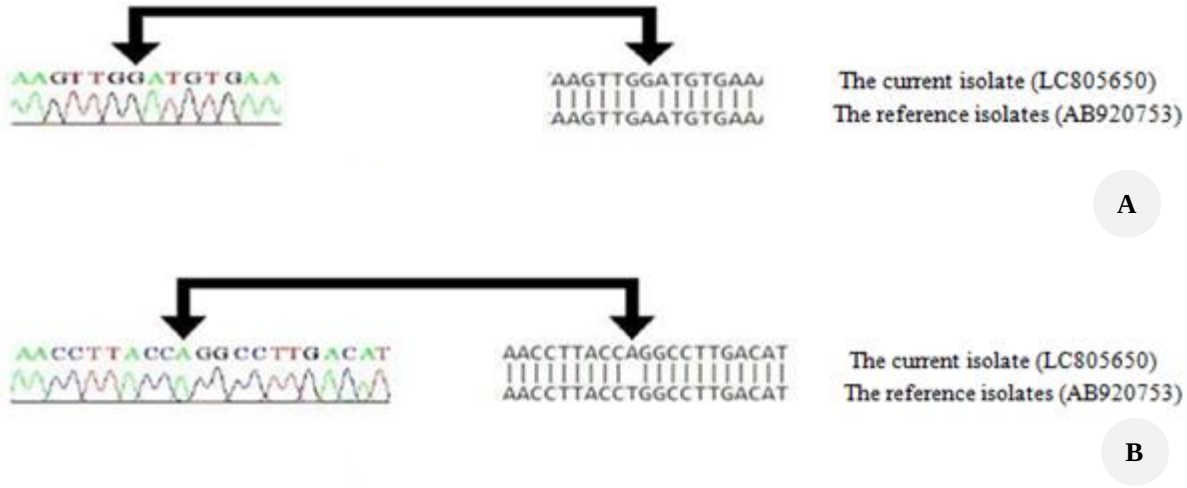

Figure 2. A. Transition mutation; B. Transversion mutation

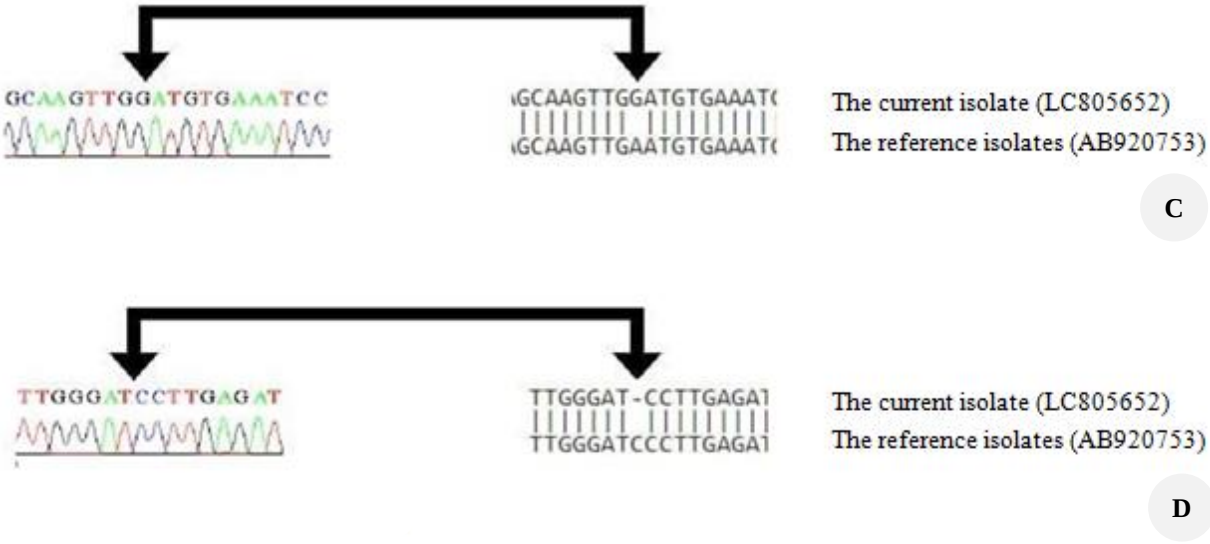

Figure 3. C. Transition mutation; D. Deletion

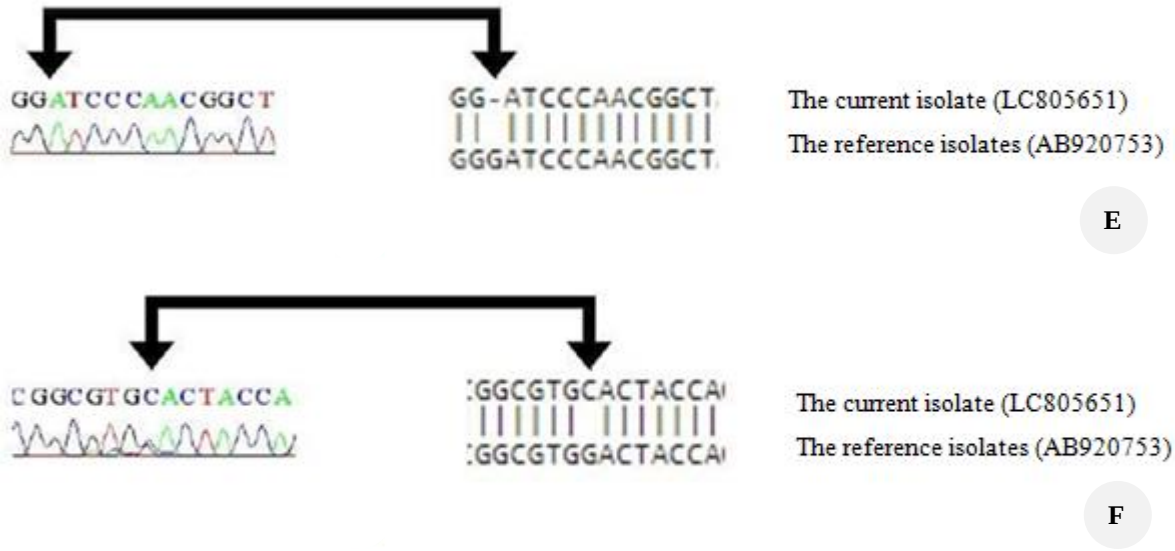

Figure 4. E. Deletion mutation; F. Transversion mutation

Table 3. Comparison of antibiotic resistance among *Pseudomonas aeruginosa*

Antibiotics	Clinical isolates	Milk isolates
	Resistance percent	Resistance percent
Amikacin (AK)	83%	100%
Ceftazidime (CAZ)	100%	100%
Ciprofloxacin (CIP)	50%	0.0%
Imipenem (IPM)	50%	0.0%
Vancomycin (VA)	100%	100%

The antibiotic resistance of isolated *P. aeruginosa*

Clinical isolates were resistant to antibiotics amikacin, ceftazidime, ciprofloxacin, imipenem, and vancomycin, with percentages of 83%, 100%, 50%, 50%, and 100% respectively, and resistance of milk isolates to these antibiotics were 100%, 100%, 0%, 0%, and 100% respectively Table 3.

Clinical isolates showed a higher rate of resistance than milk isolates to the antibiotics that are used in the study which agrees with (Mahdi 2020), who concluded in his study that *P. aeruginosa* isolates from burn swabs are more resistant than environmental isolates, also consistent with (Lerma et al. 2014; Bhuiya et al. 2018) who indicated that the resistance of *P. aeruginosa* isolated from food and the environment to antibiotics is less than the resistance of clinical isolates.

The reason may be that clinical isolates are more exposed to antibiotics than milk isolates, and thus, there is a higher chance for virulence genes to develop resistance.

Changing membrane permeability, possessing efflux pumps, producing broad-spectrum beta-lactamase enzymes, and possessing R- plasmid are all factors that help *P. aeruginosa* bacteria isolated from wounds and burns to resist antibiotics (Hong et al. 2016). However, the indiscriminate and wrong use of antibiotics to cure diseases, encourage productivity and growth, and prevent the colonization of harmful bacteria in animals led to the emergence of antibiotic-resistant bacteria (Corti et al. 2003).

Resistance of *P. aeruginosa* isolates that were isolated from milk samples to amikacin with (100%) was in agreement with (Swetha et al. 2017) who recorded full resistance (100%) for *P. aeruginosa* bacteria isolated from milk samples and (Meng et al. 2020) and inconsistent with (Shamkhi and Khudaier 2020) who documented low resistance (22.5%) for *P. aeruginosa* bacteria isolated from different samples. Also, the resistance (83%) of clinical isolates to amikacin in this study disagreed with (Mahdi 2020) and (Al-Daamy 2023). Full resistance of *P. aeruginosa* to ceftazidime in this study was agreed with (Shamkhi and Khudaier 2020) and disagreed with (Hasan et al. 2019) and (Al-Azzawi 2018). High resistance (100%) to vancomycin for this bacterium isolated from clinical and milk samples was disagreed with (Swetha et al. 2017).

The lowest resistance of *P. aeruginosa* isolates isolated from clinical samples to imipenem, with a percentage of 50%, was consistent with (Saleh et al. 2016), who reported a percentage of 55% for these bacteria that were found in milk and wound samples, and disagree with (Al-Azzawi 2018) who found that isolated *P. aeruginosa* bacteria from the wound and burn swabs resist imipenem antibiotic with percentage of 65%. The percentage of resistance (50%) to ciprofloxacin for bacteria isolated from milk or clinical samples was agreed upon (Shamkhi and Khudaier 2020).

The agreement with previous studies regarding the results of bacterial susceptibility to a specific antibiotic may be due to the similarity of the source from which they were isolated and the conditions to which they were exposed. While disagreement with other studies may belong to the difference in the source from which the bacteria were isolated, the method was used to test the bacterial susceptibility. As for the differences in the results, even the similarity of the method, the sources of isolated bacteria may be caused by differences in bacterial exposure conditions, thereby causing mutations that can increase resistance.

The reason for *P. aeruginosa* resistance to amikacin as one member of aminoglycosides is due to that the bacterium produces modified enzymes such as phosphotransferase and N-acetyl transferase (Vaez et al. 2017), in addition to the change of membrane permeability due to the occurrence of mutations (Ochoa et al. 2013).

There are two types of resistance: Intrinsic and acquired (Carlie et al. 2020); however, *P. aeruginosa* has intrinsic resistance to disinfectants and antibiotics such as ceftazidime (Lyczak et al. 2002).

Comparison of the presence of *QacE* and *QacEΔ1* genes between *P. aeruginosa* isolated from clinical and milk samples

Clinical and milk samples contain *QacE* gene with rates of 100% and 50%, respectively, and *QacEΔ1* gene with rates of 83.3% and 100%, respectively, as shown in Table 4 and Figures 5 and 6. The presence of *QacE* gene within clinical isolates in this study was in agreement with (Al-Azzawi 2018), but it was in disagreement with (Helal and Khan 2015) and (Mahzounieh et al. 2014). As for containing clinical isolates on *QacEΔ1* gene was consistent with (Mahzounieh et al. 2014), who recorded a percentage of 91.5% for this gene in *P. aeruginosa* isolates isolated from burn samples. Still, it disagreed with (Wang et al. 2007), who documented that 64.4% of *P. aeruginosa* isolates were isolated from clinical samples. The results certainly differ when comparing these genes in *P. aeruginosa* isolates of milk samples.

The difference in the presence of genes is due to the difference in the isolate source (whether it was isolated from sputum, wound, food, burn, or urine), the disinfectant to which the isolate was exposed in terms of quality and concentration, and the effect of the number of tested isolates on the study's results (Mitiku et al. 2014; Hong et al. 2016).

Relationship of *QacE and QacEΔ1* genes presence with *P. aeruginosa* resistance to multiple drugs

It was observed that milk isolate with number 3, which didn't have *QacE* gene, was Sensitive (S) to ciprofloxacin and imipenem and Resistant (R) to amikacin, ceftazidime and vancomycin compared to wound isolate with number 9 which had *QacE* gene was Resistant (R) to all included antibiotics (amikacin, ceftazidime, ciprofloxacin, imipenem, and vancomycin) in this study where it was multidrug resistance to more than three antibiotics. As for *QacEΔ1* gene, all isolates contained it except the sputum isolates with number 7, in which the susceptibility test to amikacin was Intermediate (I). Milk isolates were resistant to three antibiotics of the included antibiotics, while most of the clinical isolates were resistant to more than three antibiotics; however, multidrug resistance appeared in all *P. aeruginosa* isolates, whether isolated from milk samples or clinical samples Table 5.

The permeability of the membrane of *Pseudomonas* bacteria is low; in contrast, it has a mechanical flow pump, which qualifies it to acquire resistance to many different antibiotics (Swetha et al. 2017).

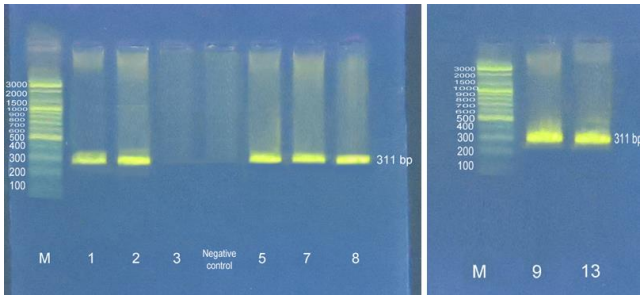

Figure 5. Gel electrophoresis of *QacE* gene

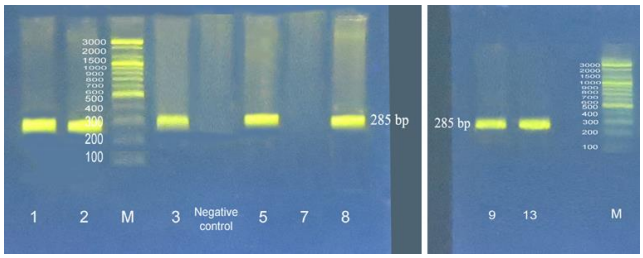

Figure 6. Agarose gel electrophoresis of PCR product for *QacEΔ1* gene, Lane M: 100 bp DNA ladder

Table 4. Comparison of *QacE* and *QacEΔ1* genes between *P. aeruginosa* isolated from clinical and milk samples

Genes	6 clinical isolates	2 milk isolates
QacE	6 (100%)	1 (50%)
QacEΔ1	5 (83.3%)	2 (100%)

Table 5. Comparison of *Pseudomonas aeruginosa* isolates in terms of sensitivity test and containing *QacE* and *QacEΔ1* genes

No. of isolate	Source of isolate	Sensitivity test					Multidrug resistance to more than three antibiotics	*QacE* gene	*QacEΔ1* gene
		AK	CAZ	CIP	IPM	VA			
1	Wound	R	R	R	S	R	+	+	+
2	Wound	R	R	S	R	R	+	+	+
3	Milk	R	R	S	S	R	-	-	+
5	Milk	R	R	S	S	R	-	+	+
7	Sputum	I	R	R	S	R	-	+	-
8	Sputum	R	R	S	R	R	+	+	+
9	Wound	R	R	R	R	R	+	+	+
13	Sputum	R	R	S	S	R	-	+	+

The resistance of Pseudomonas bacteria to many drugs is considered one of its distinctive features (Odjadjare et al. 2012; Al-Daamy 2023) due to the effect of mutations on the pores in the plasma membrane of the bacteria, which prevent the entry of antibiotics, in addition to the presence of a multi-drug efflux pump that prevents antibiotics from affecting their target if these antibiotics are pumped before they perform their work (Odjadjare et al. 2012).

The medical, environmental, and agricultural fields have suffered from a serious problem: antibiotic-resistant bacteria. Despite the spotlight on bacterial resistance that affects human health, attention has focused on food-related bacteria, especially since resistance is transmitted from pathogenic bacteria to non-pathogenic bacteria through the transmission of genes encoding resistance (Decimo et al. 2016).

Despite the presence of *QacE and QacEΔ1* genes in both isolates, clinical isolates were more resistant to multiple antibiotics than milk isolates. Differences in the expression levels of these genes may cause this. These genes are present in milk isolates but may have low expression levels compared with the high expression levels in clinical isolates.

In conclusion, the molecular methods should be adopted in the diagnosis, as other non-molecular methods may sometimes fail to diagnose. There is no point in using antibiotics such as ceftazidime and vancomycin to eliminate *P. aeruginosa*. At the same time, ciprofloxacin and imipenem have the ability to eliminate these bacteria. However, its ability decreases over time because frequent use of certain antibiotics causes mutations that increase bacterial resistance. Both types, whether *P. aeruginosa* isolated from clinical samples or milk samples, are characterized by their resistance to multiple drugs; clinical isolates are outstanding to milk isolates in this regard. Genes of *QacE and QacEΔ1* are responsible for resisting bacteria to multiple drugs. They are present in both types, whether clinical isolates or milk isolates, but their expression may be different in both types. Mutations can occur to all isolates, regardless of their source, if they are exposed to inappropriate conditions.

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