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Identification and Distribution of *Pseudomonas aeruginosa* Bacteria in Milk and Dairy Products

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Abstract | Pseudomonas aeruginosa is rarely seen on the skin or in the mucous membranes of farm animals, wild animals or pets. The numerous toxins and enzymes produced by Pseudomonas aeruginosa stimulate tissue invasion and infection. A common problem throughout the world is the contamination of milk and dairy products with pathogenic bacteria that cause decomposition. To isolate and identify Pseudomonas aeruginosa, bacteriological analysis was performed on 250 milk and cheese samples. Comprehensive investigations identified that Pseudomonas aeruginosa was found in the milk and dairy products examined in this study. The molecular confirmation and characterization of positive samples were carried out by polymerase chain reaction (PCR) targeting the Exo A gene, specific for Pseudomonas aeruginosa. The amplified genes were subjected to sequencing followed by extensive genetic analysis (available under accession number PQ836041.1). The findings showed that the gene size was 347 bp as expected and all tested 10 milk, and 17 cheese isolates showed PCR positivity. The finding of the study highlights the magnitude of the Pseudomonas aeruginosa prevalence and potential of the molecular approaches in not only confirmation but also the identification of Pseudomonas aeruginosa strains in the field samples.

Keywords | *Pseudomonas aeruginosa*, Dairy products, PCR, Bacterial isolation

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INTRODUCTION

A major concern in dairy plants is the contamination of milk and dairy products by food poisoning organisms at various processing and storage stages (Fox *et al*., 2009). More than 250 distinct ailments are brought on by consuming tainted milk products (Abrar *et al*., 2020). It has been determined that Pseudomonas species, specifically Pseudomonas aeruginosa, are responsible for many diseases. According to Ebrahimpour *et al*. (2018), this microbe is a highly significant veterinary and medical opportunistic Gram-negative. A common pathogen, Pseudomonas aeruginosa can colonize and infect a variety of species (Haenni *et al*., 2015). A specific trend in the dairy business, milk spoilage caused by psychrotrophic bacteria causes large losses for the food industry (Dogan and Boor, 2003). Before heat treatment, milk is stored at low temperatures for two to five days (De Jonghe *et al*., 2011).

Psychrotrophic microbes can proliferate and lower the quality of raw milk when it is stored at low temperatures (Xin *et al*., 2017). The most common Gram-negative bacteria that might contaminate dairy products are Pseudomonas species, which produce extracellular enzymes that are heat-stable (Cousin *et al*., 2001). Additionally,

these enzymes are resistant to UHT treatment and pasteurization (Bhunia, 2008). They can affect the quality of dairy products by altering the coagulation characteristics of milk and imparting rancid, bitter odors (Richter and Vedamuthu, 2001). Pseudomonas species. possess a high level of metabolic capacity and genetic diversity, which enables them to live in a variety of habitats, including soil, water, and air. According to Simões (2010), these traits enable them to thrive on the machinery utilized in the dairy production chain, including bulk tanks, milking machines, pipelines, and animal production environments.

The most prevalent Pseudomonas species in the dairy chain are Pseudomonas fluorescens, Pseudomonas aeruginos, and Pseudomonas putida. They produce proteolytic enzymes, which are extremely stable at high temperatures and cause milk products to spoil (March et al., 2013). It can lead to mastitis in dairy cows (Rasooli *et al*., 2018), and it can have major negative health impacts on people (Emami *et al*., 2015). Numerous cell-associated toxins, such as exo-enzyme S and exotoxin A, as well as released toxins, such as exoenzyme T and exoenzyme Y, are linked to high pathogenicity caused by *Pseudomonas aeruginosa* (Mesquita *et al*., 2013). The main reasons for a high risk of infection are poor hygiene and insufficient biosecurity.

The primary goal of the current study was to identify the abundance of common *Pseudomonas aeruginosa* in raw milk and related dairy products, such as cheese, and to exploit PCR technique to identify the species of Pseudomonas aeruginosa in cultured samples.

MATERIALS AND METHODS

Samples collections

A total of 250 milk and cheese samples were gathered from five different locations in Karbala city: the city center, Al-Hindyia, Al-Hussenyia, Al-Hur, and Ain-Altumor of these, 125 were milk samples and 125 were cheese samples. Sterile transport medium samples were used to collect the milk samples aseptically from the random samples in 10-milliliter sterile plastic vials.

Culture and identification

The samples were streaked on nutrient agar plates according to (Pawel *et al*., 2008), which were then incubated for 24 hours at 37 °C. Following Gram's staining, the distinctive suspected single colonies were sub-cultured on Citamid agar, King A agar, MacConkey agar, and blood agar. After being moved to a 1% nutritional agar slant, the pure isolates of Pseudomonas aeruginosa were kept in the refrigerator at 4 °C. A biochemical test (sugar fermentation test) was used to identify P. aeruginosa, and biochemical assays were conducted using the procedures outlined in Macfadden (2000).

PCR reaction

PCR was used to detect *Exo A* gene in the multidrug resistance bacterial strain utilizing the following primers: F: CGA CAA GAG CGA ATA CCT GGAG and R: CAA CTG GTA TTC CTC GAA ACC GTA (Axo A gene, 347 bp) (Kadhim *et al*., 2019). PCR was performed using 12 PCR water Bioneer (South Korea) and 5 µl of the template DNA. The Eppendorf Mastercycler ® thermocycler (Bioneer-South Korea) was used for the amplification process. The size-specific OXA A gene was confirmed by staining the DNA bands with ethidium bromide (Sinaclon Iran) and performing agarose gel electrophoresis (1%) of the PCR results using mM Tris-Borate EDTA (TBE) buffer at 70V for two hours. For the Bla OXA gene, a positive control was employed concurrently. Pre-denaturation at 94°C for 4 minutes, annealing at 55°C for 30 minutes, and a final extension step at 72°C were executed.

RESULTS

Culture and identification

On blood agar, the P. aeruginosa isolated from the samples formed round, mucoid, smooth colonies with β-hemolysis. (Figure 1A). Positive samples produced yellow green color colonies on Citramid Agar (Figure 1B) and flat, colorless colonies on MacConkey agar that release the smell of sweat grapes (Figure 1C) and blue-green zone due to phycocyanin production on KingA agar (Figure 1D). All Pseudomonas isolates from different sources showed positive catalase and oxidase. The result of Kligler's Iron Agar of *P. aeruginosa* was as follows: Alkaline /no change (red) No H_2S. No Gas, Cimmon citrate positive, Methyle red and Voges Proskauer negative and all of isolates negative to urease activity (Table 1, Figure 2). The VITEK2 test showed all samples with a 90% probability of *P. aeruginosa*.

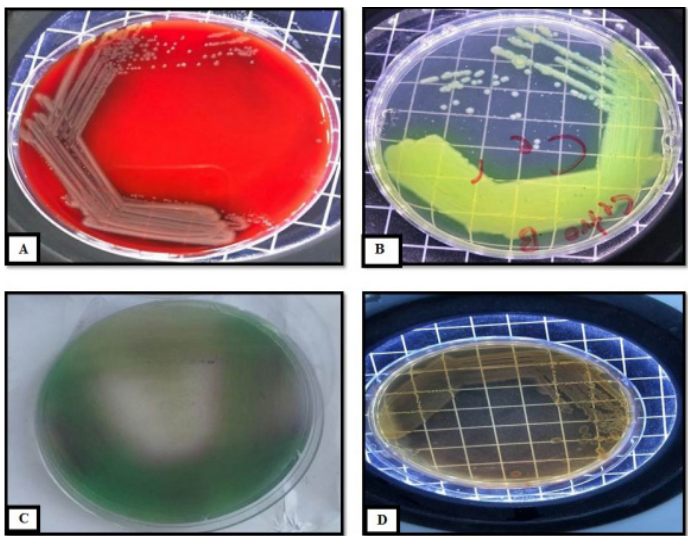

Figure 1: A: *Pseudomoas aerogenoza* in Blood agar, B: Citamid agar C: King A agar D: MacConkey agar.

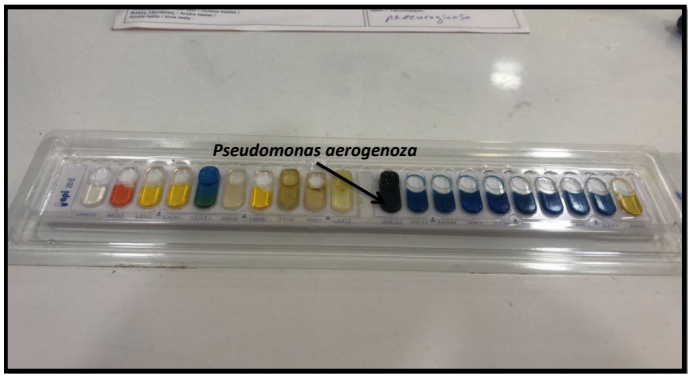

Figure 2: API 20 test for detection of *Pseudomonas aerogenoza*.

Table 1: Biochemical test of *Pseudomonas aerogenoza*.

Biochemical test	Test
Oxidase test	+
Catalase	+
Methyle red	-
Citate utilization	+
Voges proskauer	-
Urase production	-
Kigler Iron agar	K/K

Prevalence of pseudomonas on dairy product milk and cheese

The prevalence of Pseudomonas aerogenoza in raw milk was found to be 68% in the city center, which is higher than the prevalence in other regions (52%, 20%, 32%, and 36%, respectively) for Alhindyia, Al-Hussenyia, Al-Hur, and Ain-Altumor. However, the study discovered that the prevalence of Pseudomonas aerogenoza in cheese was higher in Al-Hur and Ain-Altumor (32% and 32%, respectively), and that the prevalence of cheese contamination was higher in City center, Alhindyia, Al-Hussenyia, and Al-Hur than in other locations (8%, 16% 12% and 20%, respectively).

Table 2: Distribution of Pseudomonas aerogenoza among karbala city.

Location	Raw milk		Cheese	
	No. of examined	No. positive	No. of examined	No. positive
City center	25	17 (68%)	25	2 (8%)
Alhindyia	25	13 (52%)	25	4 (16%)
Al-Hussenyia	25	5 (20%)	25	3 (12%)
Al-Hur	25	8 (32%)	25	8 (32%)
Ain-Altumor	25	9 (36%)	25	8 (32%)
Total	125	52 (41.6%)	125	25 (20%)

Molecular confirmation of *Pseudomonas aerogenoza* targeting *Exo A* gene

The amplification of the *Exo A* gene of the isolated bacteria illustrated a product size of ≈ 347 bp as expected (Figure 3).

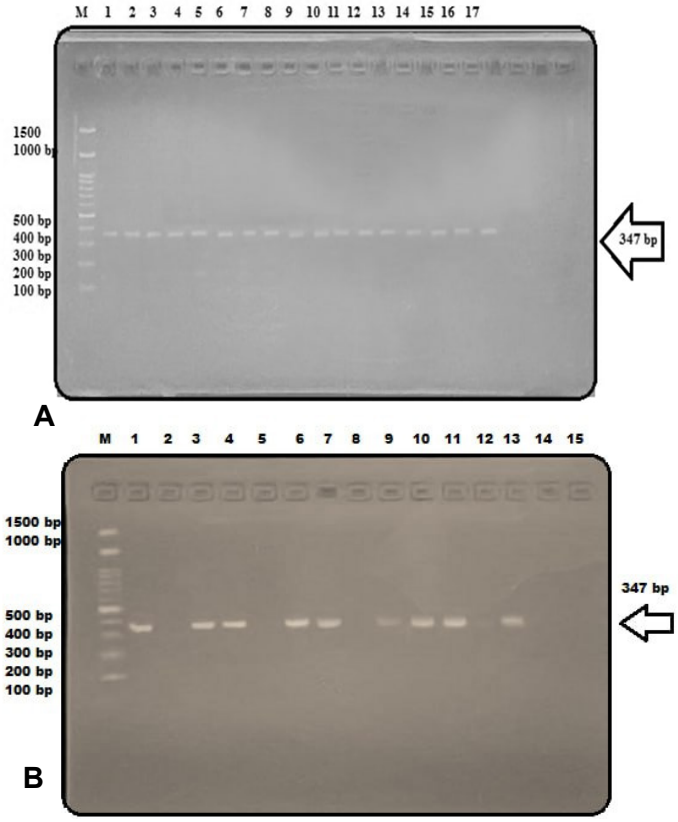

Figure 3: A: *Pseudomonas aerogenoza* in cheese. B: *Pseudomonas aerogenoza* in raw milk.

DISCUSSION

The study observed that Pseudomonas aerogenoza colonies on blood agar were smooth and circular mucoid colonies with β-hemolysis. This was in agreement with the findings of Al-Bayati *et al.* (2021), who discovered that pseudomonas colonies on blood agar were clear zones of β-hemolysis, while others displayed alpha and gamma hemolysis on blood agar bacteria for the blood hemolysis. On MacConkey agar, however, the colonies were flat, colorless, and emitted a sweat grape odor.

Additionally, the bacteria did not ferment lactose sugar, as reported by Hossain *et al.* (2013), who isolated Pseudomonas aerogenoza from cattle in Bangladesh. However, on Citramid Agar, the colonies of pseudomonas aerogenoza produced colonies that were yellow-green in color, which was consistent with (Al-Bayati *et al.*, 2021). It was discovered cultivating *P. aeruginosa* colonies on Cetrimide agar, had mucoid growth, smooth shape with flat edges and elevated center, fruity odor, and colonies that ranged in color from yellow to green. However, the blue-green zone on King A agar caused by pyocyanin production was consistent with the findings of Webster *et al.* (2015), who discovered that the bacterial isolates on King A agar were producing pyocyanin. The findings indicated that *P.*

aeruginosa was alkaline (no change, no H_2S, and no gas), positive for methyl red and Voges Proskauer, and negative for catalase and oxidase on Kligler's Iron Agar.

The study's findings regarding urease activity were in line with those of Syndya and Dhanashree (2025), who discovered that biochemical characterization was carried out using a variety of tests, including the urease, citrate utilization, methyl red, catalase, H2S production, Voges Proskauer, tryptophan deaminase (TDA), and indole tests. Additionally, the study was in agreement with the author, who was Vitek 2 compact is used as Every test card was automatically filled with a bacterial suspension after a suspension of the test organism was manually loaded into the Vitek-2 system. The cards were then incubated for six hours, with kinetic fluorescence measurements taken every fifteen minutes to monitor the growth of each well (Ling *et al*., 2022).

Pseudomonas aeruginosa was detected in 250 samples of dairy products, including 125 samples of raw milk and 125 samples of cheese. Samples taken from the City Center had the highest rate of contamination in raw milk, with 68% (17 out of 25) of the samples tested positive. Next in line were Alhindyia (13 out of 25) with 52%, Ain-Altumor (9 out of 25) with 36%, Al-Hur (8 out of 25), and Al-Hussenyia (5 out of 25) with 20%. Cheese samples, on the other hand, showed reduced contamination rates. Al-Hur and Ain-Altumor had the greatest cheese rates, at 32% (8 out of 25), followed by Al-Hindyia with 16% (4 out of 25). The City Center has the lowest rate at 8% (2 out of 25), followed by Al-Hussenyia at 12% (3 out of 25). In total, Pseudomonas aeruginosa was found in 20% of cheese samples and 41.6% of raw milk samples. According to these studies, raw milk is more likely to become contaminated in the locations under investigation than cheese (Schauer *et al*., 2021). Numerous scientific investigations have shown that raw milk can include a range of germs that cause sickness (Chang *et al*., 2024). In addition to One special disaccharide in milk is lactose. Since cow's milk contains 4.8% lactose, Pseudomonas aerogenoza may contaminate dairy products (Badawy *et al*., 2023).

One of the most researched exotoxins, exotoxin A, is released through the Type II secretion system and is encoded by the *toxA* gene. In a similar way as diphtheria toxin, it can impede the creation of proteins. Exotoxin A has been linked to both bacterial invasion facilitation and local tissue injury. Since most cases in this investigation were found in people with no known risk factors, there was no discernible correlation between the presence of risk factors and P. aeruginosa infection. Ten isolates from raw milk samples and seventeen isolates from cheese samples were found to contain the exoA gene, which has a product size of 347 bp (Akrami *et al*., 2024).

In conclusion, the study found the most common prevalence of Pseudomonas aerogenoza in dairy product milk rather than cheese as well as the risk factor exo a was found in all isolates.

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NOVELTY STATEMENT

This study provides a novel diagnostic approach by integrating phenotypic confirmation using the Vitek-2 system with molecular detection of the *exoA* gene via PCR in Pseudomonas aeruginosa isolates. This improved method of detection offers new approach for screening of this and other pathogen in field samples.

AUTHOR'S CONTRIBUTION

Bneen Najee Hassan conceived and designed the study framework. Kadhim Saleh Kadhim performed the laboratory experiments, including PCR and Vitek-2 testing and Ali Hussein Fadhil analyzed the data and interpreted the results.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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