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Study the effect of salt concentration and temperature on viability *Pseudomonas aerogenoza* isolated from Milk and Meat product in karbala City.

A Thesis Submitted to the Council of the College of Veterinary Medicine, University of Kerbala in Partial Fulfillment of the Requirements for the Master Degree of Science in Veterinary Medicine / Veterinary Public Health

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بسم الله الرحمن الرحيم

﴿وَإِنَّ لَكُمْ فِي الْأَنْعَامِ لَعِبْرَةً ۖ نُّسْقِيكُم مِّمَّا فِي بُطُونِهِ مِن بَيْنِ فَرْثٍ وَدَمٍ لَّبَنًا خَالِصًا سَائِغًا لِّلشَّارِبِينَ)

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I hereby declare that this dissertation is my original work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Karbala or other institutions.

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Dedication

Praise be to God, first and last, and then to our Prophet Muhammad and his family.

To my great homeland, Iraq

I dedicate excerpts from my humble research to my master, Imam Aba al-Fadl al-Abbas (peace be upon him).

To my eyes have never seen... my father.

To my beloved mother, the spirit of my life and the secret of my existence.

To my heart, my soulmate, my beloved, my life partner. and my support on this journey... my husband.

To my children my beloved daughter, and my companion and support, my sister Sama.

To my friends and colleagues, to all my professors, and my companions.

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Summary

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen widely distributed in the environment, including water, soil, and food products. Its presence in milk and meat poses significant public health risks due to its ability tocause sever infections, especially in immunocompromised individuals. This bacterium is known for its high resistance to antibiotics, heat, and disinfectants, making it a persistent contaminant in food processing and storage.

The contamination of dairy and meat products with *P. aeruginosa* can occur at various stages, including the period of milking, slaughtering, processing, and improper storage. Consumption of contaminated food may lead to gastrointestinal infections, septicemia, and other systemic illnesses. Moreover, some strains produce heat-stable toxins and biofilms, further increasing their threat to food safety. The study involved collecting 625 samples distributed across the holy city of Karbala, include 125 samples taken from each of the following areas: city center, Ain Al-Tamr, Al-Hindia, Al-Husseiniya, and Al-Hur. The samples were distributed as follows: 25 raw milk samples, 25 samples of cream, 25 of butter, 25 of meat, and 25 of ground meat collected from local markets.

After incubation and bacterial isolation using standard and specialized culture media, *Pseudomonas* bacteria were identified and confirmed using the VITEK system (and another method, if specified). Subsequently, an experiment was conducted to test the bacteria's heat resistance, which included exposure to four different temperatures (7°C, 21°C, 37°C and 45°C) for periods of 3 days and 7 days. Additionally, the bacteria were exposed to varying salt concentrations (2%, 5%, 7%and 10%) for the same durations.

All samples were cultured on blood agar and incubated at 37°C for 24 hours. The isolated *Pseudomonas aeruginosa* colonies exhibited as Round, mucoid, smooth colonies with β-hemolysis, Yellow-green colonies on Cetrimide Agar, Flat, colorless colonies on MacConkey agar, emitting a grape-like odor, Blue-green pigment (pyocyanin) production on King's A agar. All *Pseudomonas* isolates from different sources tested Positive for catalase and oxidase, Kligler's Iron Agar (KIA) results: Alkaline/no change (red), no H_2S production, no gas formation, Simmons citrate test Positive (utilized citrate as a carbon source), Methyl Red (MR) and Voges-Proskauer (VP): Negative, Urease activity: Negative in all isolates. The phenotypically confirmed *Pseudomonas aeruginosa* isolates were subjected to antimicrobial susceptibility testing using the VITEK-2 automated system (BioMérieux, France) following the manufacturer's protocol.

Molecular Identification of *Pseudomonas aeruginosa* by PCR Detection of Virulence genes via *exoA* and *lasB* genes, all *Pseudomonas aeruginosa* isolates were subjected to PCR-based molecular assays to detect key virulence genes.Detection of the *exoA* Gene (Exotoxin A),PCR amplification followed by agarose gel electrophoresis revealed a 347 bp DNA band, confirming the presence of the *exoA* gene. Detection of the *lasB* Gene (Elastase)Similarly, PCR and electrophoresis identified a 226 bp DNA band, verifying the *lasB* gene.These findings confirm the molecular identification of *P. aeruginosa* in the tested isolates, supporting their pathogenic potential due to the presence of critical virulence factors.

Summarizes the occurrence of *Pseudomonas aeruginosa* in dairy and meat products collected from five different regions, 25 samples were examined from five categories: raw milk, minced meat, non-minced meat,

butter, and cheese — resulting in a total of 125 samples per region and 625 samples overall. The highest contamination rate was observed in raw milk, with 52 out of 125 samples (41.6%) testing positive for P. aeruginosa. The prevalence in other products was lower: 17 positive samples (13.6%) from minced meat, 10 (8%) from non-minced meat, and 25 (20%) from cheese. Notably, none of the butter samples showed any contamination across all regions. Regionally, Al-Hur recorded the highest number of positive cases (23 out of 125; 21.6%), followed closely by the City Center and Ain-Altumor (22 positive cases each, 17.6%). Al-Hussenyia and Alhindyia showed slightly lower contamination levels, with 19 (15.2%) and 18 (14.4%) positive samples respectively.

The results of the physical tests showed that 37°C is the optimal temperature for bacterial growth and restricted growth at 7°C and 21°C during early incubation followed by complete suppression after 7 days. It was found that temperature has a significant effect on bacterial survival and at a temperature of 45 °C, no bacterial growth was observed. All samples showed noticeable bacterial growth only when exposed to a salt concentration of 2% sodium chloride, while all concentrations of 5% sodium chloride showed very little bacterial growth. At salt concentrations of 7% and 10%, no bacterial growth was observed. In Conclusion: the study found that milk and its derivatives are more contaminated with bacteria than minced and non minced meat. The study also concludes that bacteria are sensitive to temperature and salt content, with higher tolerance in low-salt environments and moderate temperature

Table of Contents

No.	Subject	Page No.
	Summary	**I**
	Table of Contents	**IV**
	List of Tables	**X**
	Table of Figures	**XI**
	Table of abbreviations	**XII**
	Chapter One: Introduction	**1-4**
1.1	Introduction	**1**
1.2	Aim of the study	**4**
	Chapter Two: Literature Review	**5-30**
2	Literature Review	**5**
2.1	The important of milk and meat in Human diet	**5**
2.2	Meat Contamination with Bacteria: Sources and Transmission to Consumers	**6**
2.3	Sources of *Pseudomonas aeruginosa* Contamination in food	**7**
2.3.1	contamination of dairy product	**7**
2.3.2	Contamination Risks to Quality and Public Health	**8**
2.4	*Pseudomonas aeruginosa* and Cheese Contamination	**8**
2.5	Contamination of Dairy Products with *Pseudomonas aeruginosa*	**10**

2.6	Puplic health and Economic important	**11**
2.7	History and Classification of *Pseudomonas aeroginosa*	**13**
2.8	Bacteriological properties of *Pseudomonas aeroginosa*	**13**
2.9	Epidemiology of *pseudomonas aeruginosa*	**16**
2.10	Host Distibution of *pseudomonas aeruginosa*	**18**
2.11	Pathways of Transmission to Consumers	**19**
2.12	Zonotic disease of *Pseudomonas aeruginosa*	**21**
2.13	Pathgenesis of *Pseudomonas aeruginosa*	**22**
2.14	Clinical signs and symptoms of pseudomonas aeruginosa	**23**
2.15	Risk of infection with *Pseudomonas aeruginosa* bacteria through consumption of contaminated meat	**26**
2.15.1	Several major routes	**26**
2.15.2	Adaptability and Growth	**26**
2.16	Preventive Measures and Risk Mitigation	**27**
2.17	The role of undercooked meat in transmitting *Pseudomonas aeruginosa* bacteria to humans	**27**
2.18	the physical and chemical properties of *pseudomonas aeroginosa*	**28**
2.18.1	Effect of temperature on growth survival of *Pseudomonas aeruginosa*	**28**
2.18.2	Effect of Salts on growth survival of *pseudomonas aeruginosa*	**30**
	Chapter Three: Methodology	**31-51**
3.	Materials	**30**

3.1	Laboratory instruments and equipment	**30**
3.2	Chemical and biological materials	**32**
3.2.1	Chemical materials	**32**
3.2.2	Biological materials	**33**
3.2.2.1	DNA extraction kit	**33**
3.3	Chemical kit used in PCR assay	**33**
3.4	Culture media	**34**
3.5	Sample collection and methods	**34**
3.5.1	Samples	**34**
3.5.2	Samples collection	**36**
3.6	Preparation of Reagents and Solutions	**36**
3.6.1	Reagents	**36**
3.6.1.1	Catalase reagent	**36**
3. 6.1.2	Oxidase reagent	**36**
3. 6.1.3	Frazier's reagent	**36**
3.6.1.4	Kovac's Reagent	**36**
3. 6.1.5	Methyl red reagent	**37**
3. 6.1.6	Voges –Proskauer reagent	**37**
3.7	Solutions	**37**
3.7.1	Gram stain solutions	**37**
3.7.2	Urea solution (20%)	**37**
3.7.3	McFarland tube standard (0.5)	**38**
3.7.4	Ethidium Bromide Solution	**38**
3.7.5	Tris-Borate-EDTA Buffer (TBE)	**38**

3.7.6	DNA loading buffer	**38**
3.8	Conventional PCR primers	**38**
3.9	Preparation of the culture media	**39**
3.9.1	King A agar (KA)	**39**
3.9.2	Blood Agar Medium	**39**
3.9.3	MacConkey agar	**40**
3.9.4	Muller Hinton agar	**40**
3.9.5	Nutrient agar	**40**
3.9.6	Nutrient broth	**40**
3.9.7	Brain heart infusion broth	**40**
3.9.8	Peptone water broth medium	**40**
3.9.9	MR-VP broth medium	**41**
3.9.10	Simmons citrate medium	**41**
3.9.11	Kligler' s iron agar	**41**
3.9.12	Urea agar medium	**41**
3.9.13	Agarose gel	**41**
3.10	Identification of *Ps aeruginosa* isolates	**42**
3.10.1	Colonial morphology and microscopic examination	**42**
3.11	Biochemical tests	**42**
3.11.1	Oxidase test	**42**
3.11.2	Catalase test	**42**
3.11.3	Indole Test	**43**
3.11.4	Methyl Red test	**43**

3.11.5	Voges – Proskauer test	**43**
3.11.6	Citrate utilization test	**43**
3.11.7	Carbohydrate fermentation and H_2S production test	**43**
3.11.8	Urease production	**44**
3.12	DNA Extraction Protocol	**44**
3.13	Lysis Protocol for Gram-negative Bacteria	**44**
3.14	PCR optimization	**46**
3.15	Agarose gel electrophoresis	**46**
3.16	Preparation of Inoculum *Pseudomonas aeruginosa*	**47**
3.17	Enumeration of Bacteria	**47**
3.18	Experiment consisted of two steps	**48**
3.18.1	Temperature	**48**
3.18.2	Sodium chloride	**49**
3.19	Dairy and meat contamination	**50**
3.19.1	Dairy and meat contamination according to Temperature	**50**
3.19.2	Dairy and Meat contamination according to salt concentration	**51**
3.20	Statistical Analysis	**51**
	Chapter Four: Results& Discussion	**52-89**
4	Results	**52**
4.1	Isolation and Identification of *P. aeruginosa*	**52**
4.1.1	Conventional identifications	**52**

4.1.2	Biochemical test	**56**
4.1.3	Confirmation with vitek system	**59**
4.2	MolecularIdentificationof*Pseudomonasaerogenoza*	**62**
4.2.1	Detection of *Pseudomonas aerogenoza* of EXO A gene	**62**
4.2.2	Detection of *Pseudomonas aerogenoza* of Las B gene	**63**
4.3	Sequencing of local Iraqi strain *Pseudomonas aeruginosa*	**66**
4.4	Survey and isolation of *Pseudomonas aerogenoza*	**68**
4.4.1	Isolation of *Pseudomonas aeroginosa* from cow milk product and meat product in different regions of karbala province	**68**
4.4.2	Enumerate of bacterial count	**75**
4.5	Experiment of the effect of temperature and salts on bacterial growth	**76**
4.5.1	Effect of Temperature on bacterial growth	**76**
4.5.2	Effect of Sodium Chloride on bacterial growth	**83**
	Chapter five. **Conclusions andRecommendations**	**90-91**
5.1	Conclusions	**90**
5.2	Recommendations	**91**
	References	**92-116**
	Appendix	
	Summery in Arabic	

List of Tables

No.	Tittle	Page No.
3.1	Instruments and equipment	**31**
3.2	Chemical materials	**32**
3.3	AddPrep Bacterial Genomic DNA Extraction Kit	**33**
3.4	Chemical kit used in PCR assay	**33**
3.5	Culture media	**34**
3.6	Primers used in this study	**39**
3.7	Thermocycler Conditions for *Pseudomnas aerogenoza*	**46**
4.1	Biochemical test of *Pseudomonas aerogenoza*	**57**
4.2	Iisolation of *P. aeruginosa* from diary product collected from different regions	**69**
4.3	Isolation of *P. aeruginosa* from meat product collected from different regions	**71**
4.4	Effect of Temperature on bacterial growth in three days	**78**
4.5	Effect of Temperature on bacterial growth in Seven days	**89**
4.6	Effect of NaCl on bacterial growth in Three days	**85**
4.7	Effect of NaCl on bacterial growth in seven days	**86**

Table of Figures

No.	Tittle	Page No.
3.1	Schematic diagram of present study	**35**
3.2	Serial dilution methods	**48**
4.1. a	*Pseudomonas aeroginosa* on Blood agar	**52**
4.1. b	*Pseudomonas aeroginosa* on Citamid agar	**53**
4.1.c	*Pseudomonas aeroginosa* in King A agar	**53**
4.1. d	*Pseudomoas aeroginosa*in MacConkey agar	**54**
4.2	API 20 test for detection of *Pseudomonas aeruginosa*	**57**
4.3	Agarose gel electrophoresis showed amplification product of 347 bp fragment of (*Exo A*) gene of *pseudomonas aeruginosa*	**63**
4.4	Agarose gel electrophoresis showed amplification product of 347 bp fragment of (*Las B*) gene of *Pseudomonas aeroginosa*	**64**
4.5	DNA sequencing of local Iraqi strain Pseudomonas aeruginosa	**67**
4.6	Isolation of *P. aeruginosa* from diary product collected from different regions	**70**
4.7	Isolation of *P. aeruginosa* from meat product collected from different regions	**72**
4.8	Inoculum strain of *Pseudomonas aeruginosa*	**76**
4.9	Effect of Temperature on bacterial growth in three days	**79**

4.10	Effect of Temperature on bacterial growth in Seven days	**80**
4.11	Growth of *Pseudomonas aeruginosa* in meat under varying temperature after 3 and 7 days of incubation	**80**
4.12	Effect of NaCl on bacterial growth in Three days	**85**
4.13	Effect of NaCl on bacterial growth in seven days	**87**
4.14	Growth of *Pseudomonas aeruginosa* in meat in different concentration of NaCl in three and seven days	**87**

List of Abbreviations

Abbreviations	Meaning
KIA	Kligler's Iron Agar
MR	Methyl Red Test
CFU	Colony Forming Unit
MIC	Minimum Inhibitory Concentration
XDR	Multi-Drug Resistant
PAB	*Pseudomonas aeruginosa* Biofilm
PCR	Polymerase Chain Reaction
VP	Voges-Proskauer Test
16S rRNA	16S ribosomal RNA gene – used for identification
TSI	Triple Sugar Iron agar

NA	Nutrient Agar
CBA	Cetrimide Blood Agar
MA	MacConkey Agar
MH	Mueller-Hinton Aga
PCR	Polymerase Chain Reaction
ExoA	Exotoxin A gene
LasB	Elastase
AIDS	Aquired immune deficiency syndrome
WHO	Whold health organization
FDA	Food and adminstartion

Chapter One
Introduction

1.1. Introduction

Dairy products such as milk are integral components of many diets worldwide, valued for their nutritional benefits and culinary versatility. However, these products can be susceptible to microbial contamination, which can lead to foodborne illnesses and spoilage **(Mafe *et al*., 2024)**. Among the various microbial contaminants, *Pseudomonas aeruginosa* is notable for its ability to survive in refrigerated conditions, making it a persistent concern in the dairy industry. The presence of this pathogen in dairy products raises significant public health concerns, necessitating a deeper understanding of its virulence factors and mechanisms of infection **(Liao *et al*., 2022)**.

Pseudomonas aeruginosa is a versatile and opportunistic pathogen that is commonly found in various environments, including soil, water, and plant surfaces. It is well-known for its ability to thrive in diverse conditions and for its resistance to many antimicrobial agents **(Zhang *et al*., 2024)**.This bacterium poses a significant threat in both clinical and agricultural settings, particularly due to its role in infections among immunocompromised individuals and its potential contamination of food products, including dairy **(Jurado-Martín *et al*., 2021)**.

The virulence of *Pseudomonas aeruginosa* is attributed to a variety of factors that enhance its ability to colonize host tissues, evade the immune response, and cause disease **(Veetilvalappil *et al*., 2022)**. Two critical virulence factors are ExoA (Exotoxin A) and LasB (Elastase),

both of which play significant roles in the bacterium's pathogenicity, while exotoxin A is one of the most important virulence factors produced by *Pseudomonas aeruginosa* **(Sánchez-Jiménez *et al*., 2023)**.

It is an A type exotoxin, where the A component is responsible for the toxic effects, and the B component facilitates the binding of the toxin to host cells. Once inside the host cell, ExoA inhibits protein synthesis by ADP-ribosylating elongation factor-2, which ultimately leads to cell death **(Valério *et al*., 2010; Schmidt, 2024)**. This mechanism not only damages host tissues but also contributes to the bacterium's ability to establish infections **(Akrami *et al.,* 2024)**.

The bacterium is particularly concerning due to its ability to form biofilms. which can protect it from environmental stressors and antimicrobial treatments **(Li *et al*., 2023)**. Biofilm formation on dairy processing equipment and storage containers can lead to persistent contamination, making it challenging to eliminate the pathogen from the production environment.Moreover, the survival of *Pseudomonas aeruginosa* in refrigerated conditions complicates food safety efforts. Traditional pasteurization methods may not completely eliminate this pathogen, especially if it is present in high numbers or if the dairy product is subsequently contaminated post-pasteurization. This resistance underscores the importance of implementing comprehensive food safety measures, including regular monitoring for the presence of *Pseudomonas aeruginosa* and its **(Zhu *et al*., 2022)**.

virulence factors in dairy product **(Atia *et al*., 2022; Li *et al*., 2023)**. This bacterium is found in various types of foods, especially those rich in moisture. Studies showed that meat, dairy products, and vegetables are the main vectors of this bacterium to consumers **(World Health Organization, 2023)**.

The contamination mechanisms in the food supply chain occur through food contamination with these bacteria occurs at several stages **(de Brito *et al*., 2022)**. During the slaughtering process, bacteria are transferred from the skin and intestines to the meat when unsterilized tools are used **(Gashe, 2022)**. In dairy plants, bacteria enter through contaminated equipment or workers. In the case of vegetables, bacteria enter through contaminated irrigation water or untreated organic fertilizers **(Ramakrishnan *et al*., 2021)**.

Meat and dairy products are among the most important routes of transmission of *Pseudomonas aeruginosa* to humans, as these products are contaminated at various stages of the food chain **(Le Berre *et al*., 2011)**. In the case of meat, the bacteria are transmitted during slaughtering and processing when unsterilized equipment is used or hygiene is neglected. In dairy products, contamination occurs either from raw milk or during manufacturing and packaging **(White *et al*., 2023)**. These contaminated products are consumed by consumers without their knowledge, especially when the bacteria are not accompanied by obvious signs of spoilage **(Siddiqui *et al* ., 2024)**.

The risk is greatest when consuming undercooked meat or unpasteurized dairy products, as the bacteria remain alive and capable of causing infection

Most cases of food poisoning occur among vulnerable groups, such as children, the elderly, and those with weakened immune systems **(Rossi *et al*., 2023)**.

1.2. Aims of study

Evaluation the contamination with *P. aerogenoza* in milk, dairy milk and meat by the fellowing:

1- Detecting *Pseudomonas* in some dairy and meat products like raw milk, cheese, butter, minced meat and non-minced mea inKarbala Province .

2- Molecular detection of some virulence gene by PCR test.

3- Analyzing the effect of different salt concentrations on the viability of *Pseudomonas*.

4- Determining the viability of *Pseudomonas* under varying temperature conditions

Chapter Two

Review of the Related Literatures

2. Review of literatures

2.1. The Importance of Milk and Meat in Human Diet

Meat and milk provide a range of important macro-nutrients such as protein, fats and carbohydrates and micro-nutrients that are difficult to obtain from plant-based foods in the required quality and quantity **(Kumar *et al*., 2020)**.

High quality protein, a number of essential fatty-acids, iron, calcium, zinc, selenium, Vitamin B12, choline and bioactive compounds like carnitine, creatine, taurine are provided by foods from terrestrial animals and have important health and developmental functions **(Alagawany *et al*., 2021)**.

Human requirements for protein have been thoroughly investigated over the years and are currently **(Teshager, 2023)**. Estimated to be 55 g per day for adult man and 45 g for woman, there is a higher requirement in various disease states and conditions of stress .These amounts refer to protein of what is termed good quality and highly digestible, otherwise the amount ingested must be increased proportionately to compensate for lower quality and lower digestibility **(Shaltout, 2024)**.

The quality of a protein is a measure of its ability to satisfy human requirements for the amino acids. All proteins, both dietary and tissue proteins, consist of two groups of amino acids - those that must be ingested ready-made, i.e. are essential in the diet and those that can be synthesised in the body in adequate amounts from the essential amino acids. Nine of the 20

food amino acids are essential for adults and ten for children **(Fernández *et al*., 2022)**.

2.2. Meat Contamination with Bacteria: Sources and Transmission to Consumers

Meat is exposed to microbial contamination at several stages of production and processing. Meat is contaminated with bacteria such as *Salmonella, E.coli, and Pseudomonas aerogenoza* through several primary sources **(Faiz *et al.*, 2024)**.

When contaminated meat is transported to processing and distribution centers, bacteria multiply rapidly if it is not stored at appropriate temperatures. Bacterial growth is stimulated when meat is left at temperatures between 4-60°C, known as the danger zone. Contaminants can alsospread during meat cutting or packaging if proper sterilization conditions are not applied. In addition, bacteria are transferred between different cuts of meat when they come into contact with each other or when the same cutting tools are used **(Smith *et al.*, 2022)**.

Meat is also stored at low temperatures to prevent bacterial growth. In addition, meat workers are educated about the importance of personal hygiene and equipment cleanliness. Finally, consumers are educated on the proper-ways to store and prepare meat at home **(World Health Organization, 2023)**.

Meat is contaminated with *Pseudomonas* bacteria through several main sources. First, these bacteria are transferred from the animal's environment before slaughter, where they are abundant in soil and water **(Watson, 2023)**.

second, meat is contaminated during slaughtering and processing when strict hygiene standards are not applied. Third, the bacteria can be transmitted through contaminated equipment and surfaces in slaughterhouses and butcher shops. These bacteria also multiply rapidly in fresh meat due to its nutrient-rich nature and high humidity **(Abass, 2021)**.

When meat is stored in unsuitable conditions, the growth of *Pseudomonas* bacteria is greatly accelerated. These bacteria have been observed to grow well at refrigeration temperatures (2-4°C), making them a threat even to chilled meat. Bacteria also secrete proteolytic and lipid-degrading enzymes, leading to changes in the organoleptic properties of meat, such as off-flavors, color, and texture. Additionally, biofilms form on the surface of meat, making decontamination more difficult **(Stellato *et al.*, 2025)**.

2.3. Sources of P*seudomonasa Aeruginosa* Contamination in Food

2.3.1. Contamination of Dairy Product

Pseudomonas aeruginosa is a common contaminant in variable environments, especially when unpasteurized milk or contaminated water is used during processing **(Dasriya *et al*., 2024)**. This bacterium thrives in humid environments, so poor equipment sterilization or cheese storage in unsuitable conditions (such as high temperatures or high humidity) increases the risk of its growth. Contamination can also occur during transportation or packaging if hygiene standards are not followed. Studies show that this bacterium is resistant to many antibiotics, making prevention crucial in the food industry **(Verraes *et al.*, 2013).**

2.3.2. Contamination Risks to Quality and Public Health

When cheeses are contaminated with *Pseudomonas* bacteria, their sensory properties, such as taste and aroma, are affected due to the secretion of proteolytic and lipid-degrading enzymes **(Al-Noman *et al*., 2022)**.

Consuming contaminated products can also lead to health problems, especially for individuals with weakened immune systems (such as diabetics or the elderly), as they can cause intestinal infections or secondary poisoning. In rare cases, the infection can spread to the bloodstream, causing serious complications. Therefore, these bacteria are classified as food safety standards **(Liguori *et al.*, 2025)**.

2.4. *Pseudomonas aeruginosa* and Cheese Contamination

The problem of *Pseudomonas aeruginosa* contamination of cheese is a major challenge facing the dairy industry worldwide **(Chowdhury *et al.*, 2024)**. This bacterium, which belongs to the Pseudomonadaceae family, is characterized by its remarkable ability to adapt to various environmental conditions, making it a major contaminant of dairy products, especially cheese **(Pedroso *et al.*, 2024)**.

The problem is most evident in soft and semi-soft cheese products, as these products provide an ideal environment for the growth and reproduction of this bacterium due to their high moisture content and rich nutritional value the danger of this bacterium lies in its remarkable ability to form biofilms on the surfaces of manufacturing and storage equipment **(Shineh *et al.*, 2023)**. Making its elimination using traditional methods extremely difficult. This bacterium is also highly resistant to many commonly used antibiotics and chemical disinfectants, further complicating the control process **(Foroughi *et al.*, 2022)**.

From a health perspective, although *Pseudomonas aeruginosa* is not considered a foodborne pathogen in the traditional sense, it may pose a risk to certain consumer groups **(Witherspoon& McCurdy, 2024)**.

People with weakened immune systems, such as cancer patients undergoing chemotherapy, AIDS patients, the elderly, or newborns, may develop serious infections from consuming products contaminated with this bacterium. Potential symptoms include severe intestinal inflammation and, in rare cases, sepsis, especially if the contaminated strain carries specific virulence factors **(Louis *et al.*, 2021)**.

To prevent the contamination of cheese with these bacteria, an integrated food safety system must be implemented across the entire supply chain. Preventive measures begin with the raw milk procurement process **(Tomasello, 2023)**. Where it must be free of contaminants, and continue through the manufacturing processes, which must be carried out under optimal sanitary conditions, to the final packaging and storage stages **(Lebelo *et al.*, 2021)**.

It is essential to implement good manufacturing practices (GMP) and hazard analysis and critical control points (HACCP) to reduce the risk of contamination. The use of modern packaging techniques, such as modified atmosphere packaging, can also help reduce the growth of these bacteria **(Ledenbach & Marshall, 2009)**.

In recent research, several innovative solutions are being explored to combat *Pseudomonas aeruginosa* in dairy products. Among these promising solutions is the use of bacteriophage **(Gliźniewicz *et al.*, 2024)**. Which selectively attack the bacteria without affecting beneficial bacteria. Recent studies also demonstrate the effectiveness of some plant extracts, such as

thyme and oregano, in inhibiting the growth of these bacteria **(Basavegowda & Baek, 2021)**.

Additionally Synergistic antioxidant and antibacterial advantages of essential oils for food packaging applications and the potential use of metallic nanoparticles, particularly those made of silver and copper, in the packaging of cheese products to prevent contamination is being investigated **(Birwal *et al.*, 2019)**.

2.5. Contamination of Dairy Products with *Pseudomonas Aeruginosa*

Milk and milk products among the most susceptible foods to microbial contamination. They are contaminated with *Pseudomonas aeruginosa* through several routes **(Singhal *et al.*, 2020)**. First, raw milk can be contaminated during the milking process due to poor hygiene or the use of unsterilized equipment. Second, products can be contaminated during manufacturing processes if strict hygiene conditions arenot followed.

Finally, contamination can occur during storage or transportation when products are stored in unsuitable conditions **(Verraes *et al*., 2013)**.

Pseudomonas aeruginosa causes spoilage of dairy products through several mechanisms. The fats and proteins in milk and butter are degraded by enzymes secreted by these bacteria. The sensory properties of the products are also altered, resulting in unpleasant odors and off-flavors. Furthermore, the nutritional value of the products is reduced due to the decomposition of the essential components. Finally, the shelf life of the products is significantly shortened due to intense microbial activity **(Crone *et al.*, 2020)**.

To protect dairy products from contamination with *Pseudomonas aeruginosa*, several preventive measures must be taken. Milk must be properly pasteurized to kill harmful microorganisms. All equipment used in manufacturing must be periodically sterilized. In addition, products must be stored at low temperatures to prevent bacterial growth. Finally, workers must be trained ingood personal hygienepractices to prevent human contamination **(Donnik *et al.*, 2022)**.

Several new methods are currently being developed to combat bacterial contamination in dairy products. The effectiveness of natural antimicrobial substances, such as medicinal plant extracts, is being tested **(Fathy *et al.*, 2023)**. The use of nanotechnology in the development of active packaging materials is also being studied.

Furthermore, new methods are being developed for the rapid detection of microbial contaminatio. Finally, manufacturing methods arebeing optimized to reduce the chances of contamination throughout all stages of production **(Breza-Boruta, 2015)**.

2.6. Puplic health and Economic Importance

Pseudomonas aeruginosa holds substantial public health and economic importance due to its role in causing a wide range of infections, especially in hospitalized and immunocompromised patients **(Ntanda, 2023)**.

It is a major cause of hospital-acquired infections such as pneumonia, urinary tract infections, and bloodstream infections, often associated with high morbidity and mortality rates **(Abban *et al.*, 2023)**.

Its resistance to multiple antibiotics makes treatment difficult, leading to prolonged hospital stays and increased use of healthcare resources. From an economic perspective, infections caused by *Pseudomonas aeruginosa* result

in significant healthcare costs related to extended treatment, isolation procedures, and the need for advanced antibiotic. Its presence in community and environmental settings also raises concerns about broader public exposure and the spread of resistant strains **(Obinna & Onyendi, 2024)**.

Pseudomonas aeruginosa is of public health and economic importance in the dairy industry due to its high resistance to environmental stresses such as elevated salt concentrations and a wide range of temperatures **(Hassan, 2025)**.

This bacterium can survive and even grow in refrigerated conditions, which are typically used to preserve dairy products, posing a challenge for food safety. Its ability to withstand sodium chloride levels allows it to persist in salted dairy products, such as cheeses and butter **(da Silva *et al.*, 2022)**.

These characteristics enable *Pseudomonas aeruginosa* to spoil dairy products, leading to economic losses through reduced shelf life, product recalls, and damage to brand reputation.

Additionally, its presence in food can pose a risk to vulnerable consumers, including infants and the elderly, emphasizing the need for strict hygiene and monitoring in dairy processing environments **(Quintieri *et al.*, 2019)**.

2.7. History and Classification of *Pseudomonas Aeroginosa*

The classification of pseudomonas aerogenoza was classified in to

Kingdom: Bacteria

Phylum: Pseudomonadota (formerly Proteobacteria)

Class: Gammaproteobacteria

Order: Pseudomonadales

Family: Pseudomonadaceae

Genus: Pseudomonas

Species: Pseudomonas aeruginosa

2.8. Bacteriological Properties of *Pseudomonas Aeruginosa*

Pseudomonas is genus of gamma proteobacteria, belonging to the larger family of pseudomonadaceae. "Pseudomonad" literally means"false unit" being derived from the Greek pseudo"false" and monas"single unit". The term "monad" was used in the early history of microbiology to denote single-celled organism, because of their widespread occurrence in soil, water and in plant seeds, the pseudomonads were observed early in the history of microbiology **(Anzai *et al.*, 2000)**.

The bactereium *P. aeruginosa* is a common bacterium that can cause disease in animals, and humans. It is found in soil, water, skin flora, and most man- made environments. It remains for long periods in soaps, sponges, sinks, oral thermometers, inhalatory equipment, dialysis fluids, tap water and clothes etc. They are extremely resistant to disinfectants and can contaminate certain compounds **(Šilha *et al.*, 2016)**.

Because it thrives on most surfaces, this bacterium is also found on and in medical equipments, including catheters, causing cross-infections in hospitals and clinics **(Tinne *et al.*, 1967)**

It is Gram-negative, aerobic, rod-shaped bacterium with unipolar motility and non-spore forming **(Hameed *et al.*, 2014)**. In liquid culture the cells occur singly, on solid culture the cells occur pairs or occasionally in short chains. The cells of some strains are surrounded by a slime substance usually surround the capsule (alginate) **(Sønderholm *et al*., 2017)**.

It is considered by many as a facultative anaerobe, as it is adapted to proliferate in conditions of partial or total oxygen depletion. This organism can achieve anaerobic growth with nitrate as a terminal electron acceptor, and in its absence, it is also able to ferment arginine by substrate- level phosphorylation. Adaptation to microaerobic or anaerobic environment is essential for certain lifestyles of *Ps. aeruginosa,* for example, during lung infection where thick layers of alginate- surrounding bacterial mucoid cells can limit the diffusion of oxygen **(Torres *et al.*, 2019)**.

Pseudomonas. aeruginosa secretes a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown) **(King *et al*.,1954)**.

Pseudomonas. aeruginosa is often preliminary identified by its pearlescent appearance and it emits sweet grape-like or tortilla-like odor *in vitro*. Definitive clinical identification of *Ps. aeruginosa* often includes identifying the production of both pyocyanin and fluorescein **(Gill & Stock, 1987)**.

The bacterium *P. aeruginosa* is an obligatory aerobe but can grow anaerobically if nitrate is available. It is found frequently as part of the microbial flora of healthy individuals. In those individuals, the gastrointestinal tract is the most frequent site of colonization, other sites such

as throat, nasal mucosa, axillae and perineum may be colonized as well **(Morrison and Wenzel,1984)**.

The organism grows readily on a wide variety of culture media in a wide range of temperature ranging from 5–42°C, but the optimum temperature is 37°C, the optimum pH is 7.4 .During the diagnosis of this organism some of cultural properties must be considered: First, Gram stain is performed, which should show Gram-negative rods with no particular arrangement, then if the *Ps. aeruginosa* grown on MacConkey agar producing colorless colonies (non- lactose ferment) **(Kushner, 1993)**.

Then, if this organism grown on Muller-Hinton agar expressing the exo-pigment pyocyanin, which is blue-green in color and colonies will appear flat, large, and oval. It also has a characteristic fruity smell. Some strains hemolysis blood of beta–hemolytic type. Clinical isolates are catalase and oxidase positive. *Ps. aeruginosa* differs from members of the Enterobacteriaceae by deriving energy from carbohydrates by an oxidation rather than fermentation, and only glucose is utilized. However, all strains give a rapid positive oxidase reaction within 30 seconds and this is a useful preliminary test for non- pigmented strains **(Kohlerschmidt *et al*., 2021)**.

Moreover, *ps. aeruginosa* is able to dehydrolyze arginine, unable to decarboxylate lysine or ornithine, unable to produce indole and H_2S, Voges–Proskauer and Methyl–Red reactions are negative. It can liquefy gelatin and utilize citrate **(Baho, 2006)**.

Furthermore, isolates from different colony types may reveal different biochemical and enzymatic activities and different antimicrobial susceptibility **(Cooper *et al*., 2003)**.

2.9. Epidemiology of *Pseudomonas Aeroginosa*

The microorganisms were frequently encountered in dairy environments, particularly as a spoilage agent rather than a direct pathogen. It is commonly found in water, soil, and moist environments **(Crone *et al.*, 2020)**. Making dairy farms and processing facilities ideal reservoirs when proper hygiene and sanitation protocols are not strictly followed.

This bacterium is psychrotrophic, meaning it can survive and multiply at refrigeration temperatures, which enhances its ability to proliferate in stored dairy products such as milk, cream, butter, and soft cheeses **(Obinn, 2023)**.

Its presence in raw milk is often associated with poor milking hygiene, contaminated water supplies, and improperly cleaned equipment. Even pasteurized milk can become contaminated post-processing due to the formation of biofilms in pipelines, storage tanks, and other surfaces tha t come into contact with the product **(Reynolds & Kollef, 2021)**.

Pseudomonas aeruginosa produces extracellular enzymes, including proteases and lipases, which degrade milk proteins and fats, leading to off-flavors, rancidity, and texture changes. Its metabolic activity often results in the production of pigments such as pyocyanin. which can cause discoloration in dairyproducts **(Tuon *et al.*, 2022)**. These spoilage characteristics significantly impact the shelf life and sensory qualities of dairy goods though it is not typically a foodborne. pathogen for healthy individuals, its antibiotic resistance and potential tocause infection in immunocompromised populations make it a public health concern.

Monitoring and controlling its presence in dairy processing environments is essential to maintaining product safety and quality **(Wang *et al.*, 2019)**.

In addition to its spoilage potential, *Pseudomonas aeruginosa* poses significant challenges in dairy production due to its ability to form strong biofilms on a variety of surfaces including stainless steel equipment, rubber gaskets, and plastic piping commonly used in milking and dairy processing systems **(Bianchi *et al.*, 2008)**.

These biofilms protect the bacteria from cleaning agents and disinfectants, allowing them to persist in the environment and continually contaminate dairy products even after routine cleaning procedures. This persistent contamination is a particular problem in large-scale dairy operations, where equipment is in constant use and cannot be frequently disassembled for complete sterilization **(Al-Orphaly *et al.*, 2021)**.

The ability of these bacteria to adapt to nutrient-poor environments means they can survive in water sources used for cleaning and cooling within milk processing facilities **(Liu *et al.*, 2023)**. Once established, *Pseudomonas aeruginosa* can colonize these systems and continually replenish the environment with new bacterial cells.

When milk or dairy products are stored for long periods, even at low temperatures, the cold- resistant nature of the bacteria allows them to multiply and cause spoilage before the product reaches the consumer. This not only results in economic losses for producers but also undermines consumer confidence in the safety and quality of dairy products **(Muller *et al.*, 2006)**.From an epidemiological perspective, *Pseudomonas aeruginosa* is not typically associated with foodborne illness outbreaks in healthy individuals,

but its presence in dairy products remains concern for immunocompromised populations, hospitalized patients, and those receiving certain medical

treatments. In rare cases, consumption of heavily contaminated dairy products can contribute to gastrointestinal disorders or infections in susceptible individuals **(Peek *et al.*, 2018)**.

Furthermore, the growing problem of antibiotic-resistant *Pseudomonas aeruginosa* strains increases the risks associated with its presence in food, as infections caused by resistant strains are difficult to treat **(Sastre-Femenia *et al.*, 2023)**.

Effective control measures include strict sanitation protocols, regular microbiological monitoring of equipment surfaces and water sources, and strict temperature control during storage and transportation **(Chandimali *et al.*, 2025)**.

It is also critical to prevent primary contamination of raw milk through proper udder hygiene, clean milking practices, and equipment maintenance. By understanding the ecology and behavior of *Pseudomonas aeruginosa* in dairy environments, producers can adopt better management practices to reduce its impact on product quality and public health **(Anversa *et al.*, 2019)**.

2.10. Host Distibution of *Pseudomonas Aeroginosa*

Pseudomonas aeruginosa is a highly adaptable, ubiquitous, and widely distributed organism among its hosts. It is considered an opportunistic pathogen, meaning it primarily infects individuals or animals with weakened immune systems or weak immune barriers. These bacteria do not typically cause disease in healthy hosts but can colonize and persist in a of environments and host organisms **(Georgieva & Dimitrova, 2016)**.

In humans, *P. aeruginosa* is often associated with hospital-acquired infections. It can infect the respiratory system, urinary tract, burns, wounds, and surgical sites. It is especially dangerous for people with chronic lung

diseases such as cystic fibrosis, as it can cause persistent infections. Patients with immunodeficiency, including those undergoing chemotherapy, organ transplants, or prolonged antibiotic treatment, are more susceptible to infection. In hospital settings, the bacteria are often spread through contaminated water, medical devices, or the hands of healthcare workers, in animals, *Pseudomonas aeruginosa* has been isolated from a variety of species, including cattle, sheep, goats, pigs, horses, dogs, and cats. It can cause mastitis in dairy cows, resulting in significant economic losses for the dairy industry. In pets, this can lead to ear infections, urinary tract infections, and skin infections, especially in animals with underlying health conditions **(Hardalo & Edberg, 1997)**.

In addition to humans and animals, the *Pseudomonas aeruginosa* bacterium is also found in plants and soil. It can colonize plant roots and has even been studied for its potential role in promoting plant growth and biological control, although its pathogenic traits limit its use in agriculture.

The bacteria are also commonly found in water systems, including hospital plumbing, hot tubs, swimming pools, and natural bodies of water.

Its abilitytosurvive in a variety of environments and on diverse hosts makes it an important environmental organism of clinical and agricultural importance **(Kiyaga *et al.*, 2022**).

2.11. Pathways of Transmission to Consumers

Contaminated meat is consumed in several ways that pose a public health risk. Undercooked meat is served in some restaurants, where the bacteria remain alive and capable of causing infection **(Alhazmi, 2013)**.

Contaminated meat is also purchased from markets when inspection procedures fail to detect contamination. Cases of food poisoning associated

with the consumption of meat containing more than 10^6 cells/g of these bacteria have been documented. Furthermore, contamination can be transferred to other foods when the same utensils or cutting surfaces are used without proper sterilization **(Kiyaga *et al.*, 2022)**.

Pseudomonas aeruginosa is a versatile and opportunistic bacterium with multiple modes of transmission, primarily through environmental exposure and contact with contaminated surfaces or materials. It is not typically spread from person to person in the same way as many other infectious agents, but rather through indirect contact and environmental reservoirs **(Kerr & Snelling, 2009)**.

One of the most common routes of transmission is via contaminated water, including tap water, swimming pools, hot tubs, and hospital water systems. The bacterium thrives in moist environments and can persist in sinks, showers, humidifiers, and even disinfectant solutions if hygiene is inadequate **(Crone *et al.*, 2020)**.

In healthcare settings, *P. aeruginosa* is often transmitted through contaminated medical equipment, such as catheters, ventilators, and endoscopes, especially when sterilization procedures are insufficient.

Patients with open wounds, burns, or compromised immune systems are particularly vulnerable to infection through these routes **(Grundmann *et al.*, 1993)**. Person-to-person transmission is rare but can occur in hospitals when proper infection control measures are not followed, especially through the hands of healthcare workers who come into contact with infected or colonized patients and then touch vulnerable individuals or surface .In community settings, it can be transmitted through contact with contaminated surfaces, such as in poorly maintained swimming pools or through the use

of contaminated personal care products like lotions or eye drops **(Botzenhart & Döring, 1993)**.

In the agricultural and veterinary context, P. aeruginosa can be transmitted to animals through contaminated water sources, bedding, or milking equipment. In dairy cows, for example, it can enter the udder through the teat canal, particularly when hygiene during milking is poor, leading to mastitis.In food products, especially in dairy environments, transmission occurs when milk or dairy equipment is contaminated by water, air, or surfaces harboring the bacterium. It can survive and multiply during cold storage, leading to spoilage and potential exposure through consumption, although it rarely causes illness in healthy individuals **(Laborda *et al.*, 2021)**.

2.12. Zonotic Disease of *Pseudomonas aeroginosa*

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen known for its resistance to multiple antibiotics and ability to cause severe infections in humans and animalsWhile primarily considered an environmental condition **(Sathe *et al.*, 2023)**.

P. aeruginosa has been increasingly recognized as a potential zoonotic pathogen, capable of transmission between animals and humans. Although *P. aeruginosa* is ubiquitous in water, soil, and plants, zoonotic transmission occurs through direct contact with infected animals or contaminated environments. Studies have identified P. aeruginosa in livestock (poultry, cattle, pigs), pets (dogs, cats), and wildlife, suggesting possible cross-species transmission .Hospital-acquired infections remain the most common, but veterinary cases highlight the risk of animal-to-human spread, particularly in immunocompromised individuals **(de Abreu *et al.*, 2014)**.

In humans, *P. aeruginosa* causes pneumonia, bloodstream infections, and chronic conditions like cystic fibrosis exacerbations. In animals, it leads to mastitis in dairy cows, otitis in dogs, and septicemia in poultry **(Eraky *et al.*, 2020)**. Shared virulence factors, such as exotoxins (ExoU) and biofilm formation, enhance pathogenicity in both hosts **(Qin *et al.*, 2022)**.

P. aeruginosa exhibits intrinsic and acquired resistance to β-lactams, fluoroquinolones, and carbapenems. Zoonotic strains from livestock often carry resistance genes (e.g., blaVIM, blaNDM), raising concerns about transmission to humans via food or occupational exposur **(Bloomfield *et al.*, 2024)**. The One Health approach is crucial in combating AMR spread across human, animal, and environmental reservoirs **(Savic *et al.*, 2024)**.

2.13. Pathgenesis of *Pseudomonas Aeroginosa*

Pseudomonas aeroginosa Rarely causes disease in humans. It is usually linked with patients whose immune system is compromised by disease or trauma **(Sathe *et al.*, 2023)**.

It gains access to these patients' tissues through the burns, for the burn victims, or through an underlying disease, like lung infection. First, *p. aeruginosa* adheres to tissue surfaces using its flagellum, pili, and exotoxin then, it replicates to create infectious critical mass; and last, it makes tissue damage using its virulence factors **(Elmassry, 2020)**.

P. aeruginosa can be transmitted to a host via fomites, vectors, and hospital workers who are potential carriers for multiply-antibiotic-resistant strains of the pathogen. Furthermore, any *Ps. aeruginosa* already present on a burn victims' skin before the injury can transform from an innocuous organism on the surface of the skin to a source of infection in the bloodstream and body tissues of the same individual **(Jaafar *et al.*, 2023)**.

Since the powerful exotoxins and endotoxins released by *P. aeruginosa* during bacteremia continue to infect the host even after being killed by antibiotics, acute disease caused by *P. aeruginosa* tends to be chronic and life-threatening **(Morin *et al.*, 2023)**.

Furthermore, most *P. aeruginosa* strains that attack compromised patients tend to be non mucoid, and even though a small number of patients infected by *P. aeruginosa* developed severe sepsis with lesions with black centers **(Reynolds & Kollef, 2021)**.

The spread of *P. aeruginosa* within host organisms is also dependent on the microorganism's elastase production and other proteases mechanisms.

Bacterial elastase and other bacterial proteases degrade the hosts proteins, including the structural proteins within membranes, disruption the hosts physical barriers against the spread of infection, elastase also assists *Ps. aeruginosa* in avoiding phagocytic antibody-mediated cytotoxicity at the site of the wound by inhibiting monocyte chemotaxis **(Khan, 2024)**.

2.14. Clinical Signs and Symptoms of *Pseudomonas aroginosa*

Pseudomonas aeruginosa infections in animals manifest through a spectrum of clinical signs that vary by species, infection site, and host immune status **(Bobrov *et al.*, 2022)**. In companion animals, otitis externa presents with foul-smelling, purulent ear discharge, often greenish, accompanied by erythema, pain, and ulceration in chronic case **(Wu et al., 2015)**. Skin infections lead to pustules, abscesses, and delayed wound healing, with distinctive blue-green pus due to pyocyanin production. Urinary tract infections cause dysuria, hematuria, and malodorous urine, while pneumonia results in coughing, dyspnea, and nasal discharge, particularly in immunocompromised or brachycephalic animals. Systemic

infections induce fever, lethargy, and septic shock, with high mortality in neonates **(Abd El-Ghany ,2024)**.

In livestock, bovine mastitis is characterized by udder swelling, watery or bloody milk, and decreased production, often linked to contaminated milking equipment. Pigs develop respiratory distress, nasal discharge, and fever when affected by P. aeruginosa pneumonia. poultry experience acute septicemia with lethargy, greenish diarrhea, and sudden death, particularly in young chicks **(Haenni *et al.*, 2015)**.

Exotic species exhibit unique presentations: birds suffer from conjunctivitis and sinusitis, reptiles develop necrotizing stomatitis
and skin ulcers, and fish show hemorrhagic septicemia with fin erosion and erratic swimming. Chronic, antibiotic-resistant infections should raise clinical suspicion for *P. aeruginosa*, warranting culture and sensitivity testing for targeted treatment. The pathogen's adaptability across species underscores the need for vigilant monitoring in both veterinary and agricultural settings to mitigate zoonotic risks **(Abd El-Ghany, 2021)**.

Pseudomonas aeruginosa foodborne infections in humans, though uncommon, can lead to gastrointestinal distress when contaminated food or water is consumed. Symptoms typically arise within hours of ingestion and include nausea, vomiting, and watery diarrhea, often accompanied by abdominal cramping and occasional low-grade fever. The presentation resembles more common forms of bacterial food poisoning, making clinical differentiation challenging without laboratory confirmation **(Urgancı *et al.*, 2022)**.

In vulnerable populations such as immunocompromised individuals, the consequences may extend beyond typical gastroenteritis. These patients risk

developing systemic infections, where the bacterium can invade the bloodstream, causing high fever, chills, and potentially life-threatening septicemia. Secondary complications like urinary tract infections or pneumonia may emerge if the pathogen disseminates from the gastrointestinal tract **(Alhazmi, 2015)**.

The bacterium thrives in various food sources, particularly dairy products that have spoiled, undercooked or improperly stored meats, and vegetables contaminated during processing **(Azad *et al.*, 2019)**. Water used in food preparation can also serve as a vehicle for transmission when sanitation is inadequate, diagnosis requires specific testing since the symptoms overlap with numerous other foodborne pathogens **(Helmy *et al.*, 2023)**.

Stool cultures are essential for definitive identification, while blood cultures may be necessary in systemic cases. Treatment becomes complicated by the organism's notorious antibiotic resistance, though targeted therapies like ciprofloxacin may be effective when susceptibility is confirmed **(Raposo *et al.*, 2016)**.

Prevention hinges on rigorous food safety practices, including proper pasteurization of dairy products, through cooking of meats, and maintenance of hygienic water supplies. For high-risk individuals, additional precautions are warranted to avoid exposure to potentially contaminated foods. The relative rarity of P. aeruginosa foodborne illness belies its potential severity in susceptible hosts, underscoring the importance of awareness among healthcare providers and food handlers alike **(Alhazmi, 2015)**.

2.15. Risk of Infection with *Pseudomonas Aeruginosa* Bacteria Through Consumption of Contaminated Meat

2.15.1. Several Major Routes

Pseudomonas aeruginosa is naturally present in soil and water and can reach meat through several major routes. This bacterium is transferred to meat during slaughtering when knives or processing equipment are ontaminated with water or contaminated surfaces **(Alves *et al.*, 2024)**. Transmission of the microbe occurs through workers who do not adhere to adequate personal hygiene procedures. This bacterium has been detected in many slaughterhouses that lack proper sterilization standards, with studies showing contamination rates as high as 15-20% in some unsealed facilities **(Ovuru *et al.*, 2024)**.

2.15.2. Adaptability and Growth

Pseudomonas aeruginosa is highly adaptable to various storage conditions, with growth observed even at refrigerated temperatures (2-4°C). It secretes proteolytic and lipolytic enzymes that degrade meat tissue, causing noticeable changes in texture and odor **(Wickramasingh *et al.*, 2019)**. Biofilms also form on the surface of meat, acting as a barrier that protects bacteria from the effects of conventional disinfectants and sterilizers. Research has shown that these films increase bacterial resistance up to 70% compared to free cells **(Strateva & Yordanov, 2009)**.

2.16. Preventive Measures and Risk Mitigation

Multiple strategies are implemented to reduce meat contamination with these bacteria. Equipment in slaughterhouses is sterilized using hot water

vapor or concentrated hydrogen peroxide solution **(Vovkotrub *et al.*,2024)**. Meat is also stored at sub-zero temperatures (-18°C) to slow bacterial growth. Modern technologies, such as packaging in modified atmospheres containing carbon dioxide, have been developed to reduce contamination rates. Data indicate that these methods have contributed to a 40% reduction in contamination cases in facilities that apply these standards **(Tsaridou & Karabelas, 2021)**.

2.17. The role of Undercooked Meat in Transmitting *Pseudomonas Aeruginosa* Bacteria to Humans

Pseudomonas aeruginosa is frequently found in raw and undercooked meat, contaminating as much as 15-30% of meat in some studies. This bacterium is isolated from samples taken from the surfaces of meat and processed meat products especially those stored under inadequate refrigeration conditions **(Qenawy *et al.*, 2024)**. The bacterium exhibits a remarkable ability to grow at refrigeration temperatures, making it a persistent threat even in refrigerated products **(Tongbram & Makroo, 2022)**.

The bacterium survives inadequate cooking, especially when meat is cooked to temperatures below 75°C. Live bacterial cells are ingested and begin secreting toxins and hydrolytic enzymes in the gastrointestinal tract. Symptoms typically appear within 6-24 hours of consumption and range from mild gastrointestinal discomfort to severe systemic infections **(Tresse *et al.*, 2003)**.

Many cases of food poisoning caused by this bacterium are diagnosed annually, especially in areas where undercooked meat is commonly

consumed. The highest incidence rates are recorded among children, the elderly, and those with weakened immune systems.

Medical reports show that 40% of cases require hospitalization, while 5% of these cases progress to severe sepsis **(Lizurej *et al.*, 2023)**. Several measures are being implemented to reduce this problem. Inspections of slaughterhouses and butcher shops are being strengthened. Food workers are being educated on the importance of hygiene and health standards. Consumers are also advised to cook meat thoroughly and not eat raw or undercooked meat. Studies show that these measures have contributed to a 35% reduction in contamination rates over the past five years **(Endale *et al.*, 2023)**.

2.18. The physical and Chemical Properties of Pseudomonas Aerogenoza

2.18.1. Effect of Temperature on Growth Survival of *Pseudomonas Aeroginosa*

Pseudomonas aeruginosa has distinct physical and chemical properties that contribute to its resilience and adaptability in various environments, including food and clinical settings **(Ambreetha *et al.*, 2024)**. It is a Gram-negative, rod-shaped, motile bacterium with a single polar flagellum that allows active movement. Colonies are typically smooth, mucoid, and can produce a distinctive blue-green pigment called pyocyanin, along with other pigments like pyoverdine, which contribute to its virulence and oxidative stress resistance **(Bhat, 2019)**.

Chemically, *Pseudomonas aeruginosa* has a versatile metabolism, enabling it to utilize a wide range of organic compounds as energy sources.

It produces several enzymes such as proteases, lipases, and elastases, which contribute to tissue degradation and food spoilage. Its cell wall structure includes lipopolysaccharides that enhance resistance to disinfectants and antibiotics **(Quintieri *et al.*, 2019)**.

Regarding the effect of temperature on growth and survival, *Pseudomonas aeruginosa* is classified as a mesophilic organism but can grow over a broad temperature range, typically from 4°C to 42°C. This wide temperature tolerance allows it to survive in refrigerated environments, making it a significant concern in cold-stored foods like dairy products **(Falih *et al.*,2024)**.

Optimal growth occurs between 35°C and 37°C, which aligns with human body temperature, supporting its role as a human pathogen. However, even at low temperatures, such as 4°C, it can survive and slowly multiply, contributing to spoilage in refrigerated foods. High temperatures above 42°C tend to inhibit its growth and eventually lead to cell death, which is why pasteurization and proper heat treatment are effective in reducing its presence in dairy products **(Quintieri *et al.*, 2019)**.

2.18.2. Effect of Salts on Growth Survival of *Pseudomonas Aeroginosa*

Pseudomonas aeruginosa shows moderate tolerance to salt, which contributes to its survival and growth in various environments, including salted foods and dairy products **(Tomaś *et al.*, 2023)**. It can grow in

environments containing up to 6–7% sodium chloride (NaCl), although its optimal growth occurs at lower concentrations. At salt levels above 7%, its growth rate decreases significantly, and survival becomes limited, especially when combined with other stress factors like low temperature or pH changes **(Ibrahim *et al.*, 2022)**.

The bacterium's ability to withstand saline conditions is linked to its adaptive stress responses, including changes in membrane composition and the accumulation of compatible solutes that help maintain cellular functions. This salt tolerance allows *Pseudomonas aeruginosa* to persist in food processing environments and in partially preserved products such as soft cheeses and salted butter. **(Wasmuth *et al.*, 2024)**.

While NaCl does not completely inhibit its growth at moderate concentrations, combining salt with other preservation methods, such as refrigeration or acidity, can be more effective in controlling its presence. This characteristic highlights the importance of integrated food safety strategies in preventing spoilage and contamination **(Tomaś *et al.*, 2023)**.

Chapter Three

Methodology

3- Materials

3.1. Laboratory Instruments and Equipments

Table (3.1) Instruments and Equipment

Instruments and Equipment	Company / origin
Autoclave	Fanem /Brazil
Benzen Burner	Memmert/Germany
Centrifuge	Gemmy/Taiwan
Different glass wares	Genx/China
Digital camera	Genx/China
Electrophoresis	Abnet, Taiwan
Hood	Memmert/Germany
Incubator	Memmert/Germany
Light microscope	Olympus/Japan
Micropipette	Slamid/England
Millipore filter papers	Proway/China
Oven	Memmert/Germany
pH-meter, Distillator	WTW/Germany
Refrigerator, Deep freeze refrigerator	Concord/Italy
Sensitive Electronic Balance	Metter-Switzerland
Shaker water bath	Memmert/Germany
Thermocycler	Memmert/Germany
UV- trans illuminator	MUV Taiwan
Vitek Compact system 2	Biometrik/ France
Water bath	Memmert/Germany

3.2. Chemical and Biological Materials

3.2.1. Chemicals

Table (3.2) Chemicals

Chemicals	***Company/country***
D-Mannose, and Ethyl alcohols, amyle–alcohol, glycerol, peptone, phenol red, iodine, ethanol, sucrose	Himedia/ India
Ethidium bromide, Na_2HPO_4, KH_2PO_4, NaCl, NH_4Cl, $MgSO_4$, glucose, Copper sulphate, DNA Loading buffer, Gelatin, $BaCl_2$, H_2SO_4, $HgCl_2$, HCL, α–naphthol, KOH, $CaCl_2$, p-dimethylamine benzyldehyde, dipyridyl	Sigma/Germany
Ethylene diamine tetracetic acid (EDTA), agarose gel	Rideal (UK)
Hydrogen peroxide (H_2O_2), potassium iodine,agar	Oxoid (UK)
Tetramethyl-p-phenylene-diamine dihydrochloride	Fluka (UK)

3.2.2. Biological Materials

3.2.2.1. DNA Extraction Kit

Table (3-3) AddPrep Bacterial Genomic DNA Extraction Kit

Diagnostic kits	***Origin***
Addbio kit	Korea

3.3. Chemical Kit used in PCR Assay

Table (3-4) Chemical Kit Used in PCR Assay

Chemicals	**Origin**
Tris-Borate-EDTABuffer (TBE buffer)	Promega/USA
TE buffer	Promega
Ladder 100bp	Biolab/Korea
Isopropanol	BDH (England)
Free nucleaseswater	Bioneer /Korea
Ethidiumbromide	Sigma /USA
Ethanol (96%)	BDH/England

3.4. Culture Media

Table (3.5) Culture Media

Materials	***Company/Origin***
Citrelmid agar, King A agar	Himedia/India
MacConkey agar, Blood agar, Nutrient agar, Muller Hinton agar, Brain heart infusion broth, Nutrient broth	Himedia/India
Simmon's citrate agar, MR-VP medium Kligler's iron agar, Peptone water medium	Oxoid (UK)

3.5 Sample Collection and Methods

3.5.1 Samples

A total of 625 samples were collected from five regions in Karbala (Ain-Altumor, Center, Al-Hindiya, Al-Hussenyia, and Al-Hur), include 125 sample from each region. The collected samples included raw milk, minced meat, non- minced meat, cheese, and butter with 25 samples from each type. Samples were transported under sterile conditions via transport medium (Nutrient broth) during aperiod of seven months from October 2024 to March 2025.

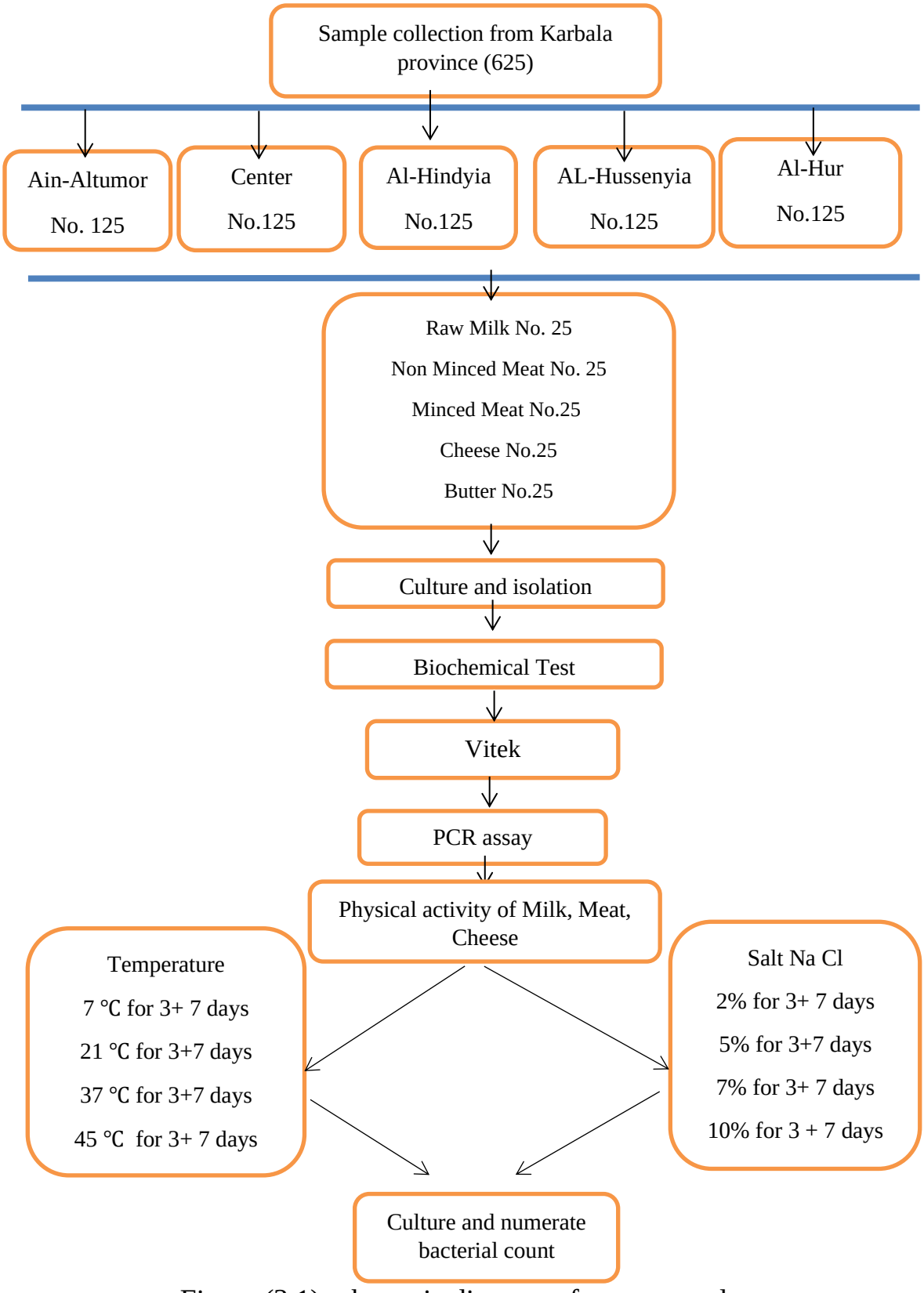


Figure (3.1) schematic diagram of present study

3.5.2 Samples Collection

Totally (625) samples were collected, each sample was taken carefully from the site of isolation and it was placed in tubes containing ready-made media to maintain the swab wet until it was taken to laboratory.

Each specimen was inoculated on King A isolation agar and Cetrmide agar, for 24 hours at 37ºC All plates were incubated aerobically in incubator at 37ºC for 24 hrs.

3.6. Preparation of Reagents and Solutions

3.6.1. Reagents

3.6.1.1 Catalase Reagent

This reagent was prepared in 3% using H_2O_2 as dilute. It was used to study bacterial ability to produce catalase enzyme **(Baron-chen et al.,1994)**.

3.6.1.2 Oxidase Reagent

This reagent was prepared by dissolving 1gm (tetramethyl-paraphenylnene–diamine–dihydrochloride) in 100ml D.W. and stored in dark bottle **(Dahlqvist, 1991)**.

3.6.1.3 Frazier's Reagent

This reagent was prepared by dissolving 15gm of mercuric chloride ($HgCl_2$) in 20ml of concentrated HCl 98%, with the addition of 100ml D.W. It was used to detect the ability of bacteria to liquefy gelatin **(DeVries *et al.*, 1996)**.

3.6.1.4 Kovac's Reagent

It was prepared by dissolving 5gm of p–dimethylamine benzyladehyde in 75ml of amyle–alcohol and then 25ml of concentrated HCl was added. It was used to detect the Indole production **(DeVries *et al.*, 1996)**.

3.6.1.5 Methyl Red Reagent

It was prepared by dissolving 0.1gm of methyl red indicator in 300ml of 95% ethanol and the volume was completed up to 500ml by D.W. This reagent was used for detection of complete glucose hydrolysis **(Dahlqvist, 1991**).

3.6.1.6. Voges–Proskauer Reagent

It was composed of two solutions A and B; these solutions used to detect the partial glucose hydrolysis were prepared according to **(DeVries *et al.*, 1996)**. and as follows:

Solution A, prepared by dissolving 5gm of α–naphthol in 100ml of absolute ethylic alcohol.

Solution B, prepared by dissolving 40gm of KOH in 100ml of D.W.

3.7. Solutions

3.7.1. Gram Stain Solutions

Those were prepared and used according to the method recommended by **(DeVries *et al.*, 1996)**. It was used to study cells morphology and their arrangement, to differentiate between Gram-negative and Gram- positive bacteria.

3.7.2. Urea Solution (20%)

It was prepared by dissolving 20gm of urea in small volume of distilled water, and completed up to 100ml distilled water, and then sterilized by Millipore filter paper. It was used in urease test for detection of urease positive bacteria **(DeVries *et al.*, 1996)**.

3.7.3. McFarland Tube Standard (0.5)

A barium-sulfate turbidity standard solution equivalent to a 0.5 McFarland standard was prepared as described by CLSI (2010), as follows: A 0.5ml of aliquot of 0.048 M $BaCl_2$ (1.175% w/v $BaCl_2.H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% v/v) with constant stirring to maintain a suspension cell density $1.5x10^8$ cell/ml.Correct density of the turbidity standard is verified by using reading the absorbance at wave 625 nm. The absorbance should be 0.08 to 0.10 for the McFarland standard. Barium-sulfate standard should be replaced or their densities verified monthly.

3.7.4. Ethidium Bromide Solution

Stock solution (5mg/ml) was prepared by dissolving 0.05gm of ethidium bromide in 10ml of D.W and stored in a dark reagent bottle **(Pospiech and Neuman, 1995)**.

3.7.5. Tris-Borate-EDTA Buffer (TBE)

Tris-OH, 0.08M, Boric acid, 0.08 M, EDTA 0.02M, were added to 800ml distilled water, the pH was adjusted to 8 and completed to one litter by Distilled water, then autoclave at 121^{o}C for 15 minutes, and stored at 4^{o}C until used **(Sambrook and Rusell, 2001)**.

3.7.6. DNA Loading Buffer

This buffer was prepared by dissolving 25mg bromophenol blue and 4mg sucrose in 10ml distilled water, and stored at 4^{o}C until used **(Sambrook and Rusell, 2001)**.

3.8. Conventional PCR Primers

The Confirmation of *Pseudomonas aerogenoza* isolates that were confirmed by conventional PCR analysis, as previously described by **(Kadhim *et al*., 2019)**.

Table (3.6): Primers used in this study.

Gene	PCR Target	PCR Product
Exo A	Forward: 5'-AAG CCT TCG ACC TCT GGA AC-3'	347 bp
Exo A	Reverse: 5'-CGT GGA TGA ACA CCT TGA TGT-3'	
Las B	Forward: 5'-CGA CAA GAG CGA ATA CCT GGAG-3'	226 bp
Las B	Reverse: 5'-CAA CTG GTA TTC CTC GAA ACC GTA-3'	

3.9. Preparation of the Culture Media

Culture media have been prepared according to the information which reported on the containers and sterilized by the autoclave and to ensure these media were not contaminated by put them in the incubator in the temperature 37°C for 24 hours and after that put them in the refrigerator. The important culture media in the diagnosis have been prepared at the following methods

3.9.1. King A Agar (KA)

It was prepared by dissolving 45gm media in the water and 20ml Bacto glycerol was added, heated to boiling to dissolve the medium completely and then autoclaved for 15 min at 121°C. This medium was used as a selective medium and for primary isolation for *Pseudomonas Aeroginosa* **(Govan, 1978)**.

3.9.2. Blood Agar Medium

It was prepared by adding 5% human blood to previously sterilized blood agar base after cooling the medium to 45°C and then poured into sterile petri dishes. This medium was used to cultivate bacterial isolates and to study the type of blood hemolysis **(Egwuatu *et al.*, 2014)**.

3.9.3. MacConkey Agar

It was prepared by adding 51.5gm of base medium to 1000ml dstilled water and sterilized in autoclave and poured in petridish. This medium contains the crystal violet to prevent grow the Gram positive and allow to grow the Gram-negative bacteria. *P. aeruginosa* appear non lactose ferment on the MacConkey agar **(Govan, 1978)**.

3.9.4. Muller Hinton Agar

It was prepared by adding 38gm in 1000ml distilled water. It was used to show pigment production by *P.aeruginosa* **(DeVries *et al.*, 1996 (McFadden, 2000)**.

3.9.5. Nutrient Agar

It was prepared by adding 28gm to 1000ml distilled water. It was used for cultivation of the bacterial isolates **(DeVries *et al.*, 1996)**).

3.9.6. Nutrient broth

Nutrient broth was prepared by dissolving 13gm in 1000ml distilled water. It was used to activation of bacteria **(DeVries *et al.*, 1996)** .

3.9.7 Brain Heart Infusion Broth

It was prepared by dissolving 37gm in 1000ml distilled water. It was used in preparation of blood culture, and it used to preserve the bacterial isolates supplemented with 15% glycerol**(DeVries *et al.*, 1996**) .

3.9.8. Peptone Water Broth Medium

This medium was prepared by dissolving 8gm peptone in 1000ml of distilled water, and then distributed into test tubes, and autoclave. It was used for demonstration of the bacterial ability to decompose the amino acid tryptophan to indole **(Green & Goldman, 2021)**.

3.9.9. MR-VP Broth Medium

It was prepared by dissolving 17gm in 1000ml distilled water, it was used to determine the ability of bacteria to produce and maintain stable acid end products from glucose fermentation **(Green & Goldman, 2021)**.

3.9.10 Simmons Citrate Medium

This medium was prepared by adding 22.8gm to 1000ml distilled water. It was used to determine the ability of bacteria to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source **(Green & Goldman, 2021)**.

3.9.11. Kligler's Iron Agar

It was prepared by adding 57.5 gm to 1000 ml distilled water. It was used to determine the ability of bacteria to utilize carbohydrate supplemented with phenol red as indicator **(Green & Goldman, 2021)**.

3.9.12 Urea Agar Medium

It was prepared by adding 10ml of urea solution (20% sterilized by Millipore filter paper) in volume of autoclave urea agar base and completed up to 100ml distilled water and cooled to 50^{o}C, the pH was adjusted to 7.1 and the medium was distributed into sterilized test tubes and allowed to solidify in a slant form. It was used to test the ability of bacteria to produce urease enzyme **(DeVries *et al.*, 1996)**.

3.9.13 Agarose Gel

The agarose gel was prepared according to the method of **(Sambrook and Rusell, 2001)** .by which 100ml of 1X TBE buffer has been taken in a beaker, 0.7gm agarose was added to the buffer, the solution was heated to boiling (using water bath) until all the gel particals dissolved, the solution

was allowed to cool down within 50-60^{O}C, and mixed with 0.5µg/ml ethidium bromide.

3.10. Identification of Pseudomonas *aeruginosa* Isolates

3.10.1 Colonial Morphology and Microscopic Examination

The grown colonies on the King A and citramid agar characterized by diffusible pigments and sweet-grape odor were selected for further diagnostic tests. The results of the following tests regarding diagnosis at *Ps. aeruginosa* were according to **(DeVries *et al.*, 1996)**.

Cultural characteristics include: colonial morphology (smooth mucoid), grape odor, its color and diffusible pigments on King A and citramid agar (bluish green or yellowish green) and inability to ferment lactose on MacConkey agar **(Green & Goldman, 2021)**. Microscopic examination includes the examination of shape (rods), gram–stain reaction, arrangement of cells with each other and rearrangment of cell

3.11. Biochemical Tests

3.11.1. Oxidase Test

A filter paper was moistened with several drops of freshly prepared oxidase reagent 1%, and then a small portion of the colony to be tested was picked up and rubbed on the moistened filter paper, changing the color to blue or purple within 30 seconds indicated for a positive test **(Baron *et al*., 1994)**.

3.11.2 Catalase Test

Few drops of 3% H_2O_2 were placed onto a portion of a pure colony on the clean, dry slide; the evolution of bubbles of gas indicates a positive test **(Baron *et al*., 1994)**.

3.11.3. Indole Test

Tubes containing peptone water were inoculated with the colony of tested bacteria and incubated at 37°C for 48 hours, and then several drops of kovac's reagent were added to the broth medium with gentle.The appearance of red ring on the surface liquid medium was regarded as a positive result**(DeVries *et al.*, 1996)**

3.11.4 Methyl Red Test

Tubes containing MR–VP broth were inoculated with the colony of tested bacteria and incubated at 37°C for 48 hours. To test culture acidity 0.5ml of methyl red reagent was added. A positive result was detected by the appearance of red color **(Baron and finegold, 1990)**.

3.11.5 Voges – Proskauer test

MR–VP medium was inoculated with the colony of tested bacteria and incubated at 37°C for 48 hours, 0.06ml of α–naphthol reagent and 0.2ml of 40% KOH solution were added. After 15 minutes, the formation of red color is indicative for the presence of acetoin (acetyl methyl carbinol) **(Baron and finegold, 1990)**.

3.11.6 Citrate Utilization Test

The surface of Simmons citrate medium was inoculated with colony of the tested bacteria and incubated at 37°C for 1–3 days. Conversion of the medium color from green to blue indicates the ability to utilize citrate as a sole carbon source **(DeVries *et al.*, 1996**).

3.11.7. Carbohydrate Fermentation and H_2S Production Test

Only the colonies grown on MacConkey agar were touched by a straight wire and inoculated on Kligler medium by stabbing the bottom of the tube and streaking the surface of slant. Fermentation was detected by a change in

the indicator phenol red to yellow. The pH changes in the bottom and the slant of medium were recorded after 18–24 hours of incubation. Gas formation is usually visualized as bubbles in the medium caused by the gas formed in the agar, black precipitate in thebottom indicates for the production of H_2S **(Baron and Finegold, 1990)**.

3.11.8. Urease Production

This enzyme can be tested by the growing of the organism in the presence of urea and testing for alkali (NH_3) production by means of a suitable pH indicator. The urea agar base was sterilized by autoclave; a deep slope of the medium was made in serial tubes then inoculated by the bacteria colonies and incubated at 37^{o}C, and examined after 4 hrs. and an overnight incubation, no tube being reported negative until after 4 days incubation. Urease positive cultures change the color of the medium to purple-pink (**Baron and Finegold, 1990)**.

3.12. DNA Extraction Protocol

The Addprep® Bacterial Genomic DNA Extraction Kit (AddBio/Korea), all phenotypically identified bacterial isolates underwent DNA extraction following the manufacturer's recommended protocol for Gram-negative bacteria appendix 1.

3.13. Lysis Protocol for Gram-Negative Bacteria

1- Centrifuge Cells: Centrifuge 1-2 mL of overnight cultured cells at 13,000 rpm for 30 seconds in a 1.5 mL tube (not provided). Discard the supernatant.
2-Add Lysis Solution: Add 200 µL of Lysis Solution and 20 µL of Proteinase K Solution (20 mg/mL) to the cell pellet, then resuspend by pipetting or vortexing.
3- Incubate: Incubate the mixture in a 56°C water bath for 10 minutes, occasionally vortexing to disperse the sample.

4- Add Binding Solution: Mix in 200 µL of Binding Solution and 200 µL of absolute ethanol by pulse-vortexing for 15 seconds, then centrifuge at 13,000 rpm for 3 minutes.

5- Transfer Supernatant: Carefully transfer 500-600 µL of the supernatant (without the pellet) to the upper reservoir of the spin column in a 2.0 mL collection tube, avoiding contact with the rim.

6- Centrifuge and Discard Flow-Through: Centrifuge at 13,000 rpm for 1 minute, then discard the flow-through and reassemble the spin column with the collection tube.

7- Washing Steps: Add 500 µL of Washing Solution 1 to the spin column, centrifuge at 13,000 rpm for 1 minute, discard the flow-through, and repeat with 500 µL of Washing Solution 2.

8- Elution: Dry the spin column by additional centrifugation at 13,000 rpm for 1 minute.

9- Transfer the spin column to a new 1.5 mL microcentrifuge tube (not provided), add 100-200 µL of Elution Solution, let stand for at least 1 minute, and then elute the genomic DNA by centrifugation at 13,000 rpm for 1 minute

3.14. PCR optimization

PCR thermocycler conditions by using convential PCR thermocycler system shown in table (3.7) (**Al-Ajmi *et al*., 2020**).

Table (3.7): Thermocycler Conditions for *Pseudomnas aerogenoza*

PCR step	Temp.	Time	Repeat
Initial Denaturation	94ºC	5min.	1
Denaturation	94ºC	25sec.	30 cycles
Annealing	63 ºC	25sec.	
Extension	72 ºC	60 Sec.	
Final extension	72 ºC	3min.	1
Hold	4 ºC	Forever	-

3.15. Agarose Gel Electrophoresis:

The agarose gel electrophoresis was done according to the procedure of **Sambrook *etal*., (1989)** as in the following steps:

The agarose (0.7gm) was dissolved in 100ml of TBE buffer by using water bath at 100ºC after that it is cooled to 50ºC, and 10 microliter of ethidium bromide solution was added to it.

Suitable amount of the agarose was poured in the tray and left to solidify.

The comb is raised from the agarose and the tray was transferred to the electrophoresis chamber and covered with TBE buffer.

Ten microliters of the DNA extract are mixed with 3 microliters of the loading buffer, and it placed in the agarose wells.

The electrophoresis process was carried using low voltage (about 6 volt/cm and by passing 20 milliamper) for 1-2 hours.

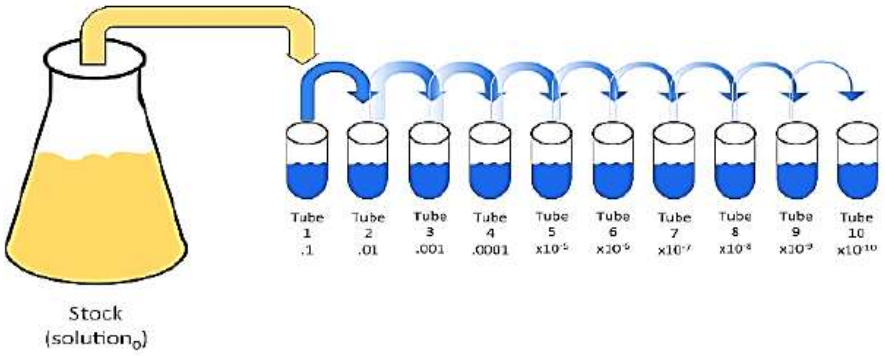
The bands of the plasmid DNA were detected by UV-trans illuminator at a wave length (302) nanometer.

3.16. Preparation of Inoculum *Pseudomonas Aeruginosa*

A loopful of brain heart infusion broth was utilized to culture the *Pseudomonas aeruginosa* bacteria. This broth was incubated at 37°C for 18 hours before being collected with phosphate-buffered saline (PBS, pH 7.2). The sample was rinsed with PBS and centrifuged at 2000 rpm for 5 minutes at 7 °C, discarding the supernatant. The resulting pellet was then resuspended in sterile PBS (pH 7.2). The suspension was diluted and adjusted to a concentration of (1×10^{10} cells/mL) CFU/mL using viable plate count methods **(Kim *et al.*, 2012)**.

3.17. Enumeration of Bacteria

The bacterial count was determined from various dilutions of the experiment. 100 microliters of bacterial was transferred to 99.9 ml of Phsphate buffer saline, Bacteria present in the samples were counted using a bacteria counting device, as shown in figure (3-2). The samples were then plated on King A agar, and colonies that developed on the agar plates were counted after incubation for 24 hours at 37°C **(Highsmith & Abshire, 1975)**.



Figure(3-2): Serial dilution method (Rashad, 2017).

$$\text{Dilution factor} = \frac{1\ ml}{1\ ml + 9\ ml} = \frac{1}{10} = 10^{-1}$$

Figure (3.2.): Serial dilution methods **(Boukouvalas et al., 2019)**

3.18. Experiment Consisted of Two Steps

The experiment involved two steps:

3.18.1. Temperature

After isolating *Pseudomonas aeruginosa* from milk, cheese, butter, meat and macerated meat, the bacteria were cultured on King A agar and incubated at the specified temperatures as outlined in the below.

7 ℃ for 3 and 7 days

21 ℃ for 3 and 7 days

37 ℃ for 3 and 7 days

45 ℃ for 3 and 7 days

3.18.2. Sodium Chloride

The Sodium Chloride solutions were prepared according to the specified concentrations, and the bacteria were diluted in them before being cultured on King A agar and incubated at the designated temperatures, as outlined in the following tables. To assess the individual effect of each salt concentration on *Pseudomonas aeruginosa*, 50 µl of an 18–24-hour broth culture was inoculated into 20 ml of the respective treatment tubes. The inoculated tubes were vortexed and maintained at room temperature (25 ºC) for the specified contact times. At intervals of 1 hour, 12 hours, 24 hours, and 96 hours, 1 ml from each tube was serially diluted in 9 ml of sterile 0.1% peptone water, and viable counts were determined using the spread plate method. The experiment was conducted in triplicate. Bacterial counts were recorded as CFU/ml or CFU/g. At the end of the incubation period, only petri plates containing *Pseudomonas aeruginosa* colonies ranging between 15 and 150 were considered for bacterial enumeration. Plates with more than 150 colonies were classified as "too many to count" (TMTC) and were excluded from analysis, while those with fewer than 15 colonies were labeled "too few to count" (TFTC). Colonies on each plate were counted using a Quebec colony counter the bacteria were cultured on King A agar and incubated at the specified Sodium cholride as outlined in the below and figure (3.2)

2% NaCl to 3 and 7 days

5% NaCl to 3 and7 days

7% NaCl to 3 and 7 days

10% NaCl to 3 and 7 days

3.19. Dairy and Meat Contamination

3.19.1. Dairy and Meat Contamination According to Temperature

Samples of meat, minced meat, cheese, butter, and milk were each inoculated with *Pseudomonas aeruginosa* 10^6. A quantity of 5 grams or 5 milliliters of each food product was used for the inoculation. The inoculated samples then incubated under controlled conditions at temperatures of 4°C, 7°C, 21°C, 37°C, and 45°C for periods of three and seven days. Following incubation, microbial analysis was carried out to evaluate bacterial growth. Each sample was subjected to serial dilution up to 10^6 to estimate the concentration of bacteria. From each dilution, aliquots were plated on King A agar and incubated under appropriate conditions to allow colony development. Colony-forming units were observed and counted to determine whether the bacterial population had increased, decreased, or remained stable over the incubation period. The presence or absence of colonies in the agar plates was used to assess whether each food product served as a suitable medium for the growth of *Pseudomonas aeruginosa* under the tested conditions. Uninoculated control samples were maintained in parallel to rule out external contamination and confirm the accuracy of the observations

3.19.2. Dairy and Meat Contamination According to Salt Concentration

To evaluate the effect of sodium chloride concentration on the growth of Pseudomonas aeruginosa, samples of meat, minced meat, cheese, butter, and milk were inoculated with 10^6. Each product was supplemented with varying concentrations of NaCl, specifically 2%, 5%, 7%, and 10%. Following the addition of salt, 5 grams or 5 milliliters of each treated product were inoculated and incubated at 37°C for periods of three and seven days. After incubation, the samples were subjected to serial dilution, reaching up to a 10^6 dilution level, to estimate bacterial concentration. Aliquots from each dilution were plated onto King A agar and incubated under appropriate conditions to allow colony development. The number of colony-forming units was recorded to assess the effect of salt concentration on bacterial survival and proliferation. Growth patterns were analyzed to determine whether higher salt concentrations inhibited bacterial development or allowed for persistence and adaptation. Uninoculated control samples with corresponding salt levels were maintained to confirm the sterility of the products and exclude the possibility of external contamination.

3.20. Statistical Analysis

All data were analyzed using one way ANOVA through the General Linear Models procedure , one way anova and Chi-Square test , an interactive calculation tool for chi-square tests of goodness of fit and independence (**Preacher, 2001**)

Chapter Three: Methodology…….3

Chapter Four

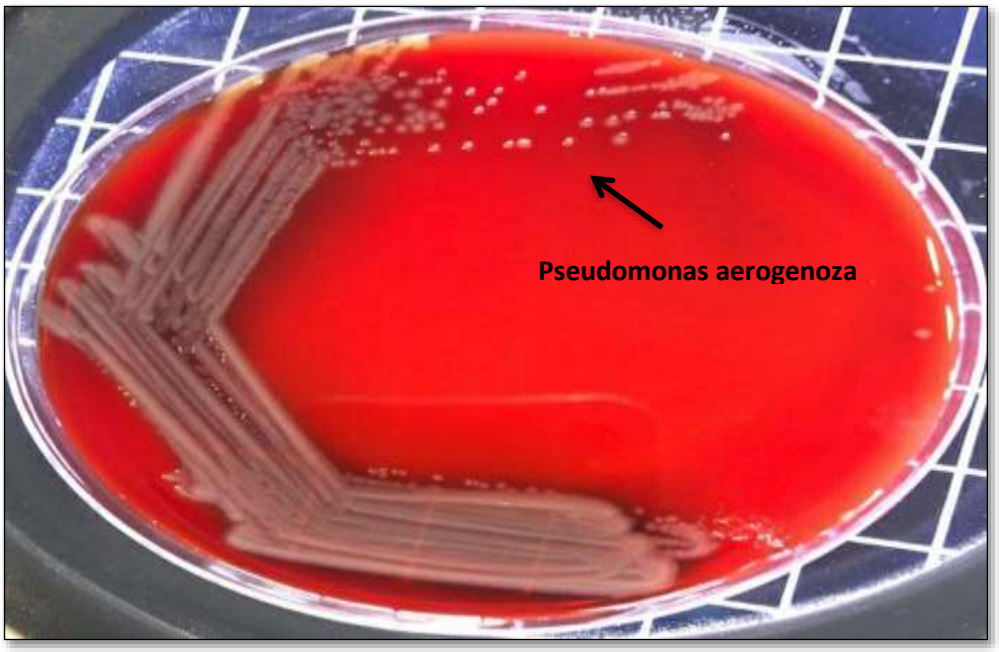
Results and Discussion

4. Results

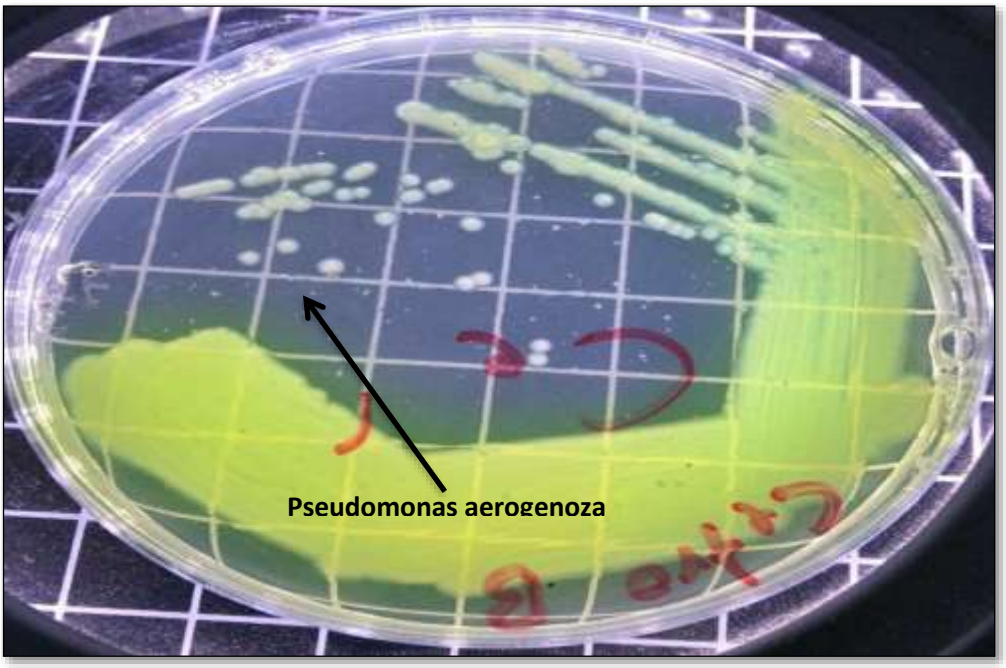
4.1. Isolation and Identification of *P. aeruginosa*

4.1.1. Conventional Identifications:

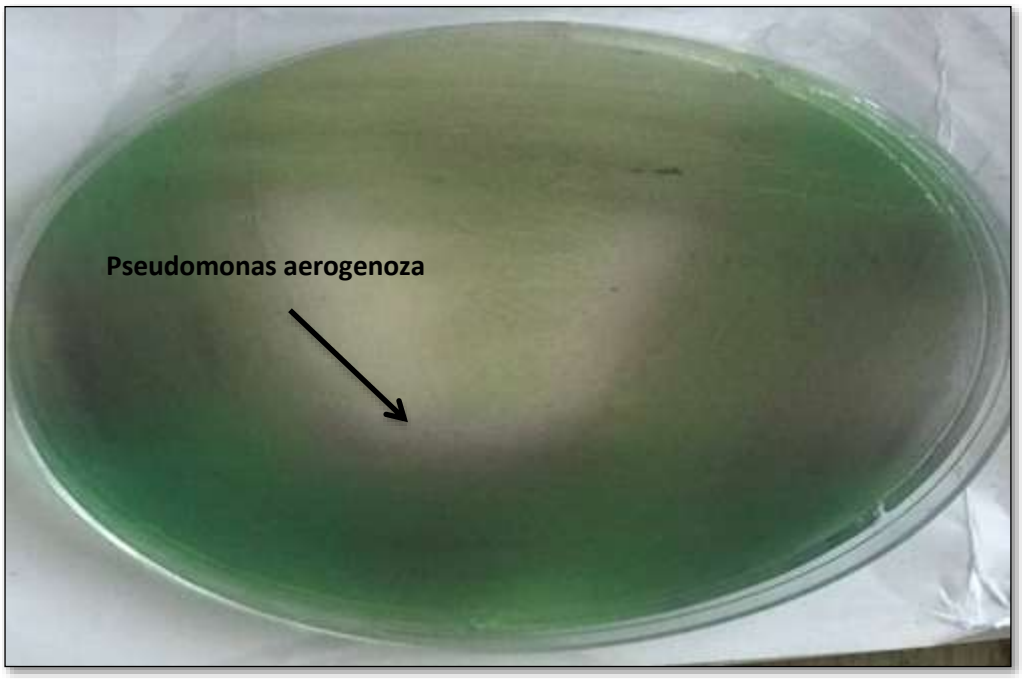
All samples were cultured on blood agar after being incubated 37^0C for 24 hours, the P. aeruginosa isolated from the samples formed round, mucoid, smooth colonies with β-hemolysis (Figure 4.1A) and produce yellow green color colonies on Citramid Agar (Figure 4. 1B) and flat, colorless colonies on MacConkey agar that release the smell of sweat grapes (Figure 4.1C) and blue-green zone due to phycocyanin production on KingA agar (Figure 4.1D).



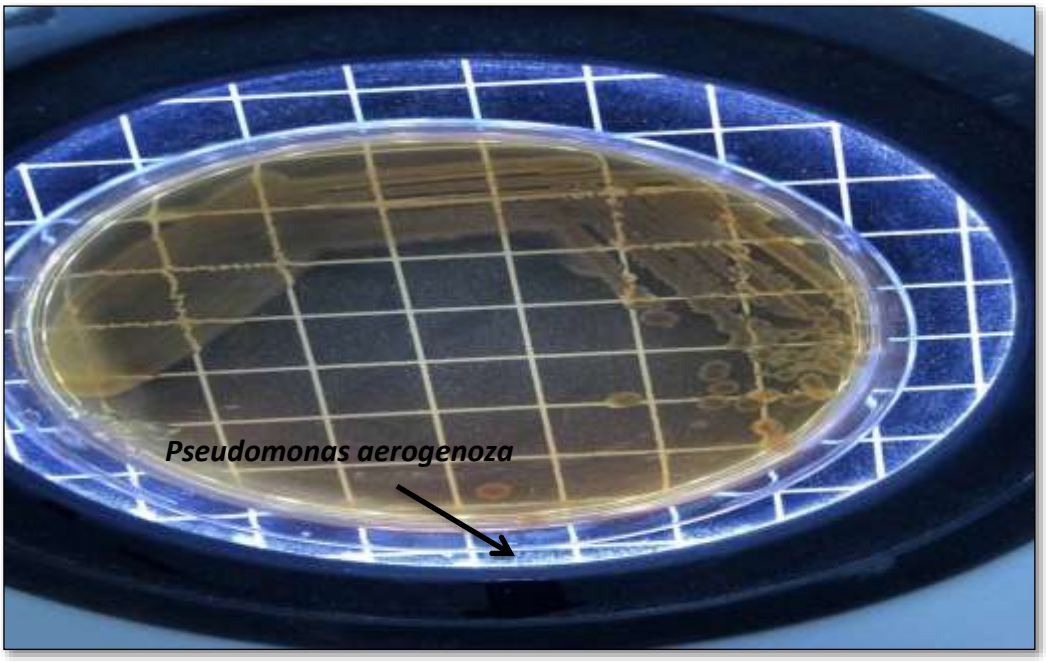
Figure(4-1A): *Pseudomonas aerogneza* round mucoid smooth colonies on Blood agar



Figure(4-1B): Pseudomonas aerogneza yellow green colonies on Citamid agar



Figure(4-1C): Pseudomonas aerogenoza blue green zone colony in King A agar



Figure(4-1D): *Pseudomoas aerogenoza* with flat and colorless colony in MacConkey agar

Pseudomonas aeruginosa was cultured on blood agar, and complete beta-hemolysis was observed, indicating the bacteria's high capacity to produce erythrocyte-lysing factors. This hemolysis pattern is attributed to the production of compounds such as hemolysin and pyrocyanin, which contribute to significant cellular damage and enhance the virulence of the bacteria in clinical infections, especially in immunocompromised patients **(Lee & Zhang, 2024)**.This behavior has been documented in numerous studies examining the characteristics of *Pseudomonas aeruginosa* in various clinical settings, the sample was then transferred to Stermid agar, which is selectively used for the detection of *Pseudomonas aeruginosa* due to its compounds that inhibit the growth of most other microorganisms.

The bacteria were found to grow well and produce a yellow-green fluorescein stain, an important indicator for the initial identification of *Pseudomonas aeruginosa* **(Okezie *et al.*, 2021)**. This pigment—along with pyrocyanin—reflects the unique metabolic capabilities of bacteria to produce pigments with antimicrobial activity against other microorganisms, enhancing their survival in multi-organism environments **(Sakhtah *et al.*, 2023)**.

Pseudomonas aeruginosa was cultured on MacConkey agar, a selective, differential medium used to detect the bacteria's ability to ferment lactose **(SEKHI, 2024)**.Colonies wereobserved togrow without lactose fermentation and to appear transparent or pale, a characteristic of this gram-negative, non-fermenting bacterium. This metabolic characteristic reflects its ability to survive in nutrient-poor environments, enhancing its susceptibility to chronic infection in the human host **(Martínez-Alcantar *et al.*, 2023)**.

The sample was then cultured on King A agar, a medium specifically designed to stimulate the production of pyocyanin, one of the phenazine pigments produced by *Pseudomonas aeruginosa* **(Abdelaziz *et al.*, 2023)**. The colonies displayed a distinctive grassy green color, indicating the bacteria's ability to efficiently produce this pigment. Pyocyanin is an effective factor in enhancing bacterial virulence, as it contributes to the generation of reactive oxygen species, weakens the host immune response, and has a direct toxic effect on human cells **(Cazares-Garcia *et al.*, 2024)**. 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 **(Al-Bayati *et al.* (2021)**.

Neverthe less, 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 pigment.

4.1.2. Biochemical Test

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 H2S. No Gas, Cimmon citrate positive, Methyle red and voges proskauer negative and all of isolates negative to urease activity (Table 4.1, figure 4. 2).

Table (4-1): Biochemical test of *Pseudomonas aerogenoza*

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

Figure (4-2): API 20 test for detection of *Pseudomonas aerogenoza*

All *Pseudomonas aeruginosa* isolates showed positive activity for both catalase and oxidase; results were consistent with the known characteristics of this bacterium. The oxidase test is crucial in distinguishing *Pseudomonas aeruginosa* from other Gram-negative bacilli as it detects cytochrome c oxidase activity, which contributes to electron transfer reactions. Catalase activity also enhances the bacteria's ability to withstand oxidative stress by degrading hydrogen peroxide, an indicator of their high environmental adaptability, especially in chronic infections and stressful environments **(Alves *et al.*, 2023)**.

When Kligler's iron agar test was performed, all isolates showed an alkaline/no change (red/red) reaction pattern, with a complete absence of hydrogen sulfide or gas production. This reflects the non-fermentative nature of the bacteria and their inability to metabolize glucose or lactose via pathways that lead to acid or gas production. This reaction demonstrates the extent to which *Pseudomonas aeruginosa* relies on aerobic pathways for energy generation are unlike many other Gram-negative bacilli that exhibit clear fermentation patterns **(Tavares *et al.*, 2024).**

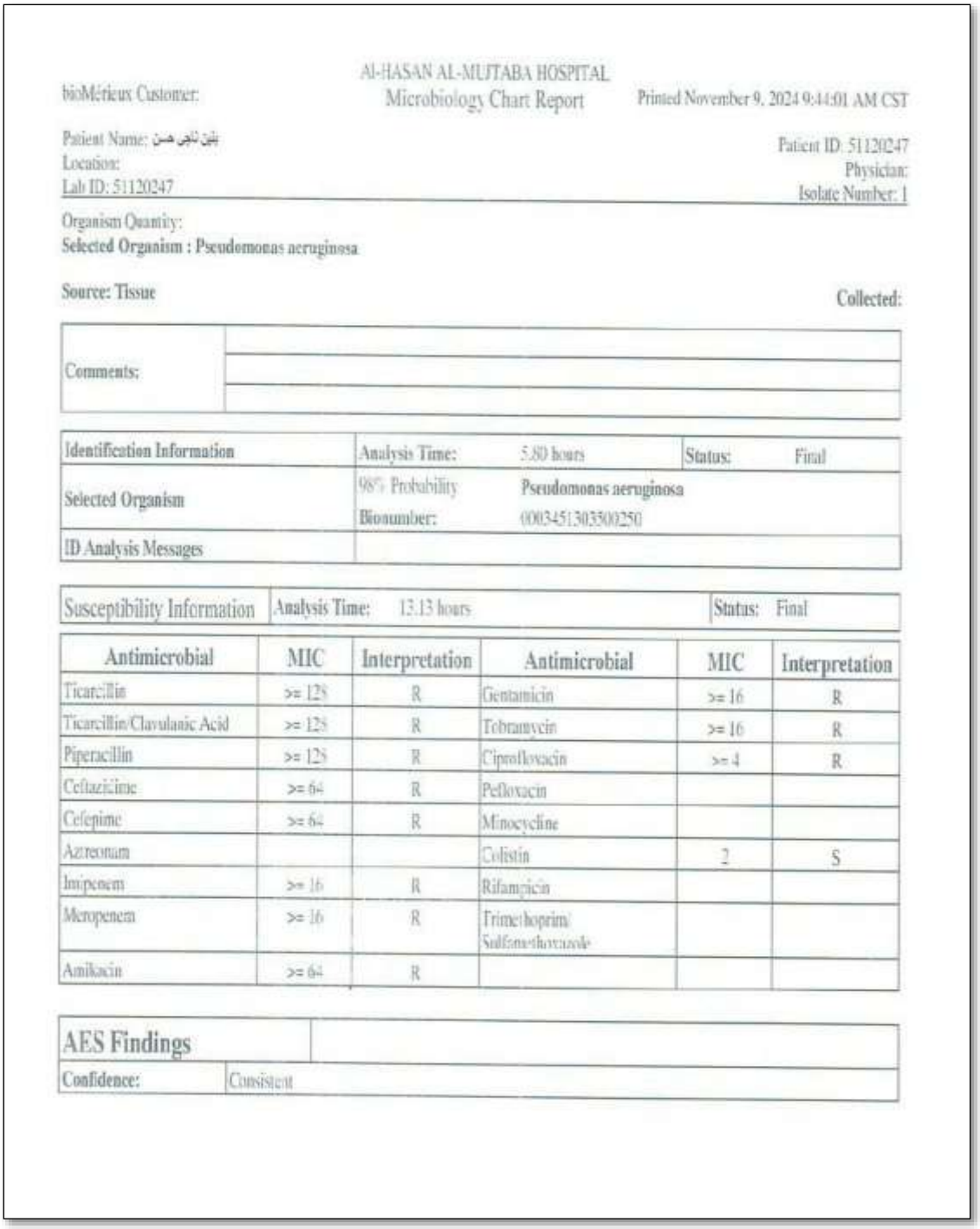
The Simon citrate test yielded a positive result, indicating *Pseudomonas aeruginosa*'s ability to utilize citrate as its sole carbon source **(Ismail *et al.*, 2020)**. This characteristic distinguishes this species from a series of identification tests and reflects its high metabolic flexibility, enabling it to grow in limited nutrient conditions. The isolates also yielded negative results in the methyl red and Fuegis-Proskauer tests, indicating that they do not produce strongly acidic or neutral compounds such as acetoin during glucose metabolism, further strengthening their classification as non-fermenters (**Sharma & Banerjee, 2023)**.

Finally, all isolates tested negative for the urease test, indicating their inability to hydrolyze urea into ammonia and carbon dioxide a feature that helps distinguish *Pseudomonas aeruginosa* from other bacteria such as Proteus and Klebsiella, which test positive for this test. This reaction pattern is used as a key component of routine microbial identification systems **(Mekonnen *et al.*, 2021)**.

The finding of the study regarding urease activity were in line with those of **Liaqat *et al.* (2019)**, 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.

4.1.3. Confirmation with Vitek System

Phenotypically confirmed *Pseudomonas aeruginosa* isolates were tested using the disc diffusion method with the Vitek-2 system (BioMérieux, Marcy L'Étoile, France). The testing procedure followed the manufacturer's protocol as outlined in the documentation provided with the device. The results are presented in Figures 4 and 5, the VITEK2 test showed all samples with a 90% probability of *p. aeruginosa*. and the study providence by statistical analysis



AL-HASAN AL-MUTABA HOSPITAL

bioMérieux Customer: Microbiology Chart Report Printed November 9, 2024 9:44:01 AM CST

Patient Name: بلين ناجي حسن Patient ID: 51120247
Location: Physician:
Lab ID: 51120247 Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: Tissue **Collected:**

Comments:	

Identification Information	Analysis Time:	5.80 hours	Status: Final
Selected Organism	98% Probability	**Pseudomonas aeruginosa**	
	Bionumber:	0003451303500250	
ID Analysis Messages			

Susceptibility Information	Analysis Time: 13.13 hours	Status: Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ticarcillin	>= 128	R	Gentamicin	>= 16	R
Ticarcillin/Clavulanic Acid	>= 128	R	Tobramycin	>= 16	R
Piperacillin	>= 128	R	Ciprofloxacin	>= 4	R
Ceftazidime	>= 64	R	Pefloxacin		
Cefepime	>= 64	R	Minocycline		
Aztreonam			Colistin	2	S
Imipenem	>= 16	R	Rifampicin		
Meropenem	>= 16	R	Trimethoprim/ Sulfamethoxazole		
Amikacin	>= 64	R			

AES Findings	
Confidence:	Consistent

The VITEK 2 Compact system (Biomérieux, France) was used to identify the bacterial isolates, and the system demonstrated that all isolates were *Pseudomonas aeruginosa* with high accuracy.

This automated system, based on biochemical analysis, provides rapid and reliable results for the classification of Gram-negative bacteria, which is consistent with the nature of *Pseudomonas aeruginosa* as non-fermenting, enzymatically active organisms. These results are consistent with those of **Gurung *et al.* (2023)**, who reported that the VITEK 2 device exceeded 95% in identifying *Pseudomonas aeruginosa* when compared to molecular diagnosis using gene sequencing, confirming its effectiveness in clinical settings. However, other studies, such as **(Kamal *et al*. 2014)**. have indicated that the VITEK 2 system's accuracy in identifying *Pseudomonas aeruginosa* was greater than 95% when compared to molecular diagnosis using gene sequencing, confirming its effectiveness in clinical settings.Indicated a slight possibility of misdiagnosis in the case ofatypical isolates or those undergoing physiological changes due to environmental stress or exposure to antibiotics.

This may require the use of supporting techniques such as MALDI-TOF or polymerase chain reaction (PCR) to enhance diagnostic accuracy. These results enhance the reliability of relying on automated systems such as the VITEK 2 in clinical diagnostic laboratories, especially in cases of complex infections. However, integration with conventional tests and clinical information remains essential to achieve an accurate and comprehensive diagnosis.

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)**.

4.2. Molecular Identification of *Pseudomonas aerog*inosa

4.2.1. Detection of *Pseudomonas Aeroginosa* of EXO A Gene

Pseudomonas aerogenoza isolates were identified by molecular assay for detection of the *exoA* gene was performed using PCR for all samples. PCR-positive samples were subsequently analyzed by agarose gel electrophoresis, which revealed a DNA band of approximately 347 bp, indicating the presence of the target gene associated with *Pseudomonas aeruginosa*.

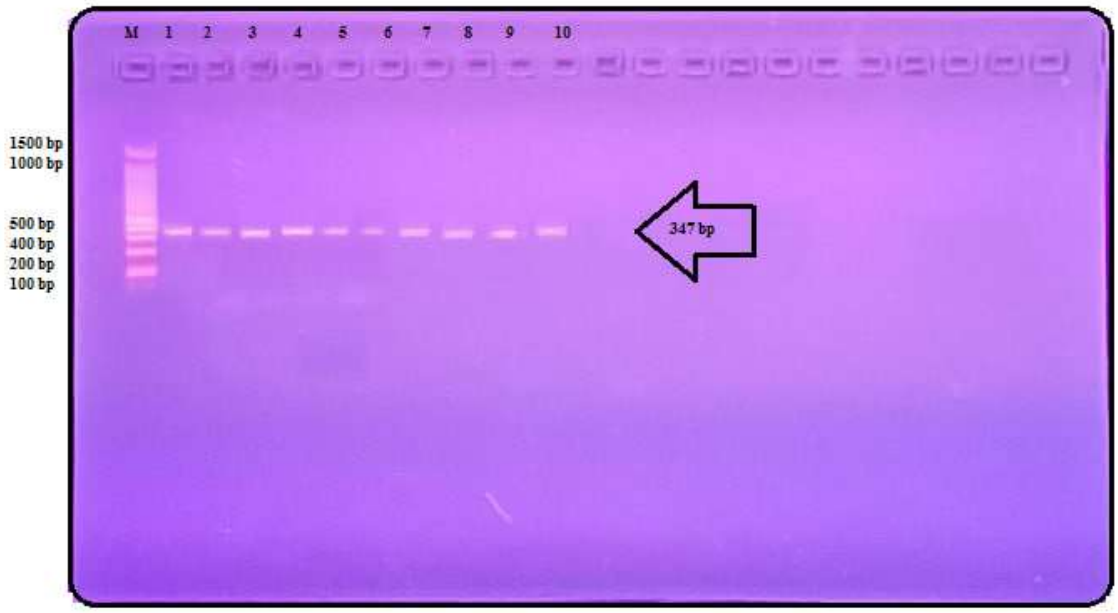


Figure (4.3): Agarose gel electrophoresis showed amplification product of 347 bp fragment of (*Exo A*) gene of pseudomonas aerogenoza

4.2.2. Detection of *Pseudomonas Aeroginosa* of Las B Gene

Pseudomonas aerogenoza isolates were identified by molecular assay for detection of the *lasB* gene was performed using PCR for all samples. PCR-positive samples were subsequently analyzed by agarose gel electrophoresis, which revealed a DNA band of approximately 226 bp, indicating the presence of the target gene associated with *Pseudomonas aeruginosa*.

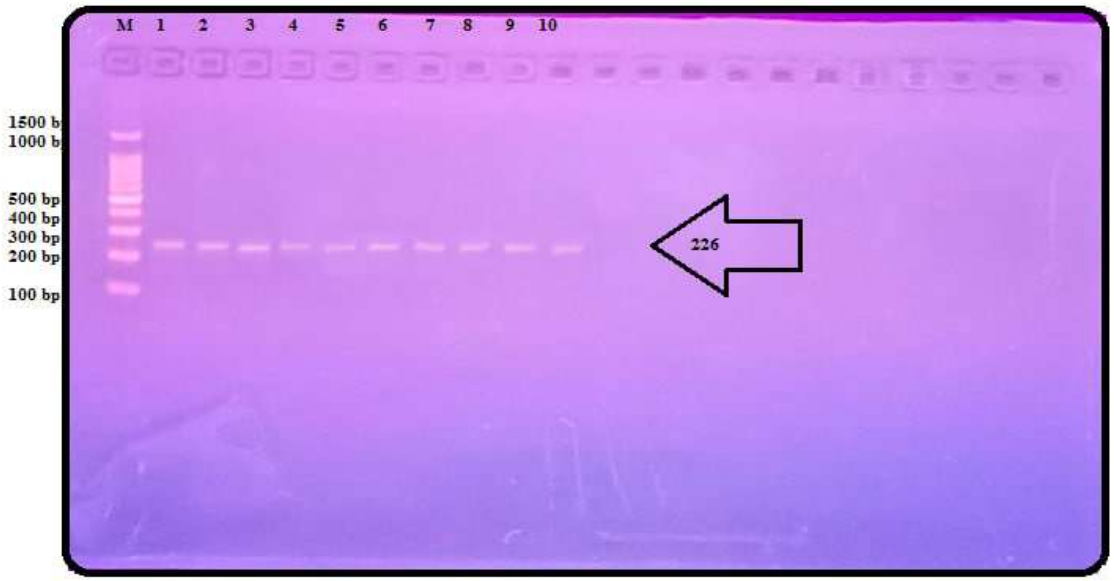


Figure (4-4): Agarose gel electrophoresis showed amplification product of 226 bp fragment of (*Las B*) gene of *Pseudomonas aerogenoza*

The ExoA gene was successfully amplified from *Pseudomonas aeruginosa* isolates, and the amplification yielded a product approximately 347 base pairs in size, which is consistent with the known gene size of this gene encoding Exotoxin A. ExoA is considered one of the most important virulence factors secreted by *Pseudomonas aeruginosa*, inhibiting protein synthesis in host cells through ADP-ribosylation of the elongation factor EF-2, leading to cell death. A recent study by **(Al-Daraghi & Abdullah, 2013)**, confirmed that the presence of this gene in clinical isolates is associated with higher infection rates and greater severity, especially in cases of pneumonia and surgical infections.

On the other hand, amplification of the LasB gene, with a product approximately 226 base pairs in size, was also confirmed. This gene is responsible for the production of elastase B, a protease enzyme that plays an important role in tissue destruction and the degradation of immune proteins such as IgG and C3. Isolates containing this gene exhibit a high capacity to induce local damage to host tissues and increased vascular leakage, which contributes to the spread of infection. A study by **Fernández-Piñar *et al.* (2024)**, demonstrated that LasB is an important indicator of *Pseudomonas aeruginosa* virulence, especially in the context of cutaneous and corneal infections and chronic respiratory infections.

The simultaneous detection of the ExoA and LasB genes indicates that these isolates possess a complex set of virulence factors, enhancing their ability to cause severe and persistent infections, especially in immunocompromised patients. The combination of direct toxicity and tissue-lytic activity makes these factors vital targets for vaccine and antibody development studies. A recent study by **Habib *et al.* (2024)**, indicated that the co-presence of these genes is a predictive indicator of the development of multidrug resistance and the ability to form biofilms.

The ExoA and LasB genes were detected in isolates obtained from food products including milk, cheese, and minced meat, indicating the presence of virulent strains of *Pseudomonas aeruginosa* in food chains. This discovery is indicative of the potential microbial risk in untreated or improperly stored foods, especially since these genes encode toxins and enzymes that are effective in destroying cells. In a study by**Azimi *et al.* (2016),** *P. aeruginosa* was isolated from raw cow's milk and a significant

proportion of isolates were found to contain the ExoA gene, suggesting the potential transmission of the bacteria through milking practices or poor storage conditions. The study concluded that molecular detection of this gene could be an effective monitoring tool for assessing the safety of dairy products. A recent study by **Hemmati *et al.* (2024)**, demonstrated the presence of the LasB gene in *P. aeruginosa* isolates from ground beef. The presence of this gene has been linked to an increased ability to produce proteolytic enzymes that affect food texture and facilitate bacterial translocation within the body after consumption. The study suggested that the presence of LasB in food-borne strains is an indicator of virulence potential, even outside of a clinical setting.

4.3. Sequencing of Local Iraqi Strain *Pseudomonas Aeruginosa*

The 16S rRNA gene of the bacterial isolated *Pseudomonas aeruginosa* strain was amplified and sequenced Sanger. The retrieved sequence was BLASTed in NCBI nucleotide database and exhibited high similarity to reference strains of *Pseudomonas aeruginosa* reported worldwide. The alignment validated that the isolate contained conserved areas with reference P. aeruginosa strains, demonstrating genetic uniformity with internationally reported sequences. These findings, together with collection data, showed that the isolate under study is a member of the global clone of *Pseudomonas aeruginosa* figure (4-9) and we obtained accesiion number on gene ba nk website (PQ836041.1) appendix 2

;

Figure (4-5): DNA sequencing of local Iraqi strain *Pseudomonas aeruginosa*

In this study, *Pseudomonas aeruginosa* was successfully isolated from dairy and meat products in Karbala City, Iraq, and identified through 16S rRNA gene sequencing. The results were consistent with those reported by **Altaai *et al*. (2014),** who utilized the 16S rRNA gene for the differentiation of *P. aeruginosa* from other *Pseudomonas* species isolated from clinical and environmental samples.

The 16S rRNA gene was confirmed as a reliable molecular marker for the accurate identification of *P. aeruginosa*, as demonstrated in both studies. Similar genetic profiles were observed, reinforcing the specificity of this method for distinguishing *P. aeruginosa* from closely related species. The findings further support the applicability of 16S rRNA sequencing in diverse sample types, including food products, as highlighted in the present work,

and clinical/environmental samples, as reported by **Amutha & Kokila (2014)**.

4.4. Survey and Isolation of *Pseudomonas aeroginosa*

4.4.1. Isolation of *Pseudomonas Aerogenoza* from Cow Milk Product and Meat product in Different Regions of Karbala Province.

A total of 375 dairy product samples, including raw milk, butter, and cheese, were examined across five different locations in Karbala City to detect the presence of Pseudomonas aeruginosa. The overall contamination rate was found to be 20.5%, with raw milk showing the highest positivity rate at 41.6%, followed by cheese at 20%, while no contamination was observed in butter samples. Among the locations studied, the City Center recorded the highest prevalence, where P. aeruginosa was detected in 68% of raw milk samples and 8% of cheese samples. In contrast, Al-Hussenyia exhibited the lowest contamination rate, with only 20% of raw milk and 12% of cheese samples testing positive. No butter samples tested positive in any location, indicating a possible inhibitory effect of the butter matrix or processing conditions on the survival of the bacterium. The statistical analysis indicated a significant difference among the contamination rates across different locations ($\chi^2 = 29.382$; DF = 16; P = 0.021), suggesting that geographical or handling variations may influence contamination levels. It was observed that raw milk was the most frequently contaminated product across all locations, highlighting the potential risk of consuming unpasteurized milk.

Cheese samples from Al-Hur and Ain-Altumor also showed a notably higher rate of contamination at 32%, implying possible issues in post-processing hygiene or storage conditions. The findings underscore the need for improved sanitary measures and routine microbial monitoring, particularly in the handling and distribution of raw milk and cheese in Karbala City table (4.2).

Table (4-2): isolation of *Pseudomonas Aeroginosa* from diary product collected from different regions

Location	Raw Milk		Butter		Cheese		Total / Location	
	No. of examined	No. positive	No. of examined	No. positive	No. of examined	No. positive	No. of examined	No. positive
City center	25	17 (68%)	25	0 (0)	25	2 (8%)	75	19 (25.3%)
Alhindyia	25	13 (52%)	25	0 (0)	25	4 (16%)	75	17 (22.6%)
Al-Hussenyia	25	5 (20%)	25	0 (0)	25	3 (12%)	75	8 (10.6%)
Al-Hur	25	8 (32%)	25	0 (0)	25	8 (32%)	75	16 (21.3%)
Ain-Altumor	25	9 (36%)	25	0 (0)	25	8 (32%)	75	17 (22.6%)
Total	125	52 (41.6%)	125	0 (0)	125	25 (20%)	375	77 (20.5%)
Statistical analysis	X2= 14.35, DF= 4, p=0.006		Ns		X2= 8, DF= 4, p=0.09		-	

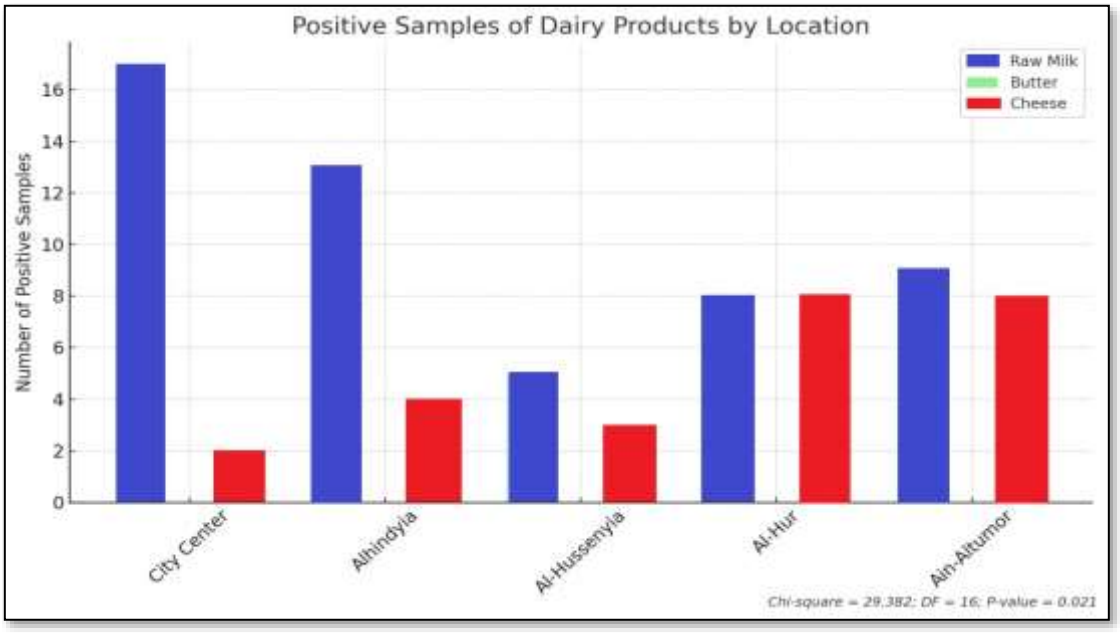


Figure (4-6): isolation of *Pseudomonas Aeroginosa* from diary product collected from different regions

A total of 250 meat samples, comprising 125 minced and 125 non-minced samples, were collected and examined for the presence of *Pseudomonas aeruginosa* across five locations in Karbala City. An overall contamination rate of 10.8% was observed. Minced meat showed a higher rate of contamination at 13.6%, while non-minced meat exhibited a lower rate of 8%. Among the surveyed locations, Al-Hussenyia presented the highest prevalence, where 24% of minced and 20% of non-minced meat samples were found to be contaminated, indicating a notable microbial burden in this area. On the other hand, Alhindyia reported the lowest contamination rate, with only 4% of minced meat samples testing positive and no detection in non-minced samples. A moderate level of contamination was detected in samples from Al-Hur and Ain-Altumor, with minced meat in these locations showing higher positivity compared to their non-minced counterparts. The

City Center recorded a low overall contamination rate of 6%, with only minced meat samples affectedThe statistical analysis revealed a significant difference in contamination rates among locations ($\chi^2 = 12.45$; DF = 8; P = 0.021), suggesting variability in handling, storage, or sanitation practices. The higher contamination rate in minced meat could be attributed to the increased surface area and handling involved in the mincing process, which may facilitate bacterial proliferation. These findings emphasize the importance of enforcing strict hygiene controls during meat processing and retail handling to reduce the risk of *P. aeruginosa* contamination in meat products distributed in Karbala City table (4.3).

Table (4-3): isolation of *Pseudomonas Aeroginosa* from meat product collected from different regions

Location / Product	Minced Meat		Non-Minced Meat		Total / Location	
	No. of examined	No. positive	No. of examined	No. positive	No. of examined	No. positive
City center	25	3 (12%)	25	0 (0)	50	3 (6%)
Alhindyia	25	1 (4%)	25	0 (0)	50	1 (2%)
Al-Hussenyia	25	6 (24%)	25	5 (20%)	50	11 (22%)
Al-Hur	25	3 (12%)	25	4 (16%)	50	7 (14%)
Ain-Altumor	25	4 (16%)	25	1 (4%)	50	5 (10%)
Total	125	17 (13.6%)	125	10 (8%)	250	27 (10.8%)
Ststistical analysis	X2= 4.49, DF= 4, p=0.34		X2= 11.95, DF= 4, p=0.017		-	

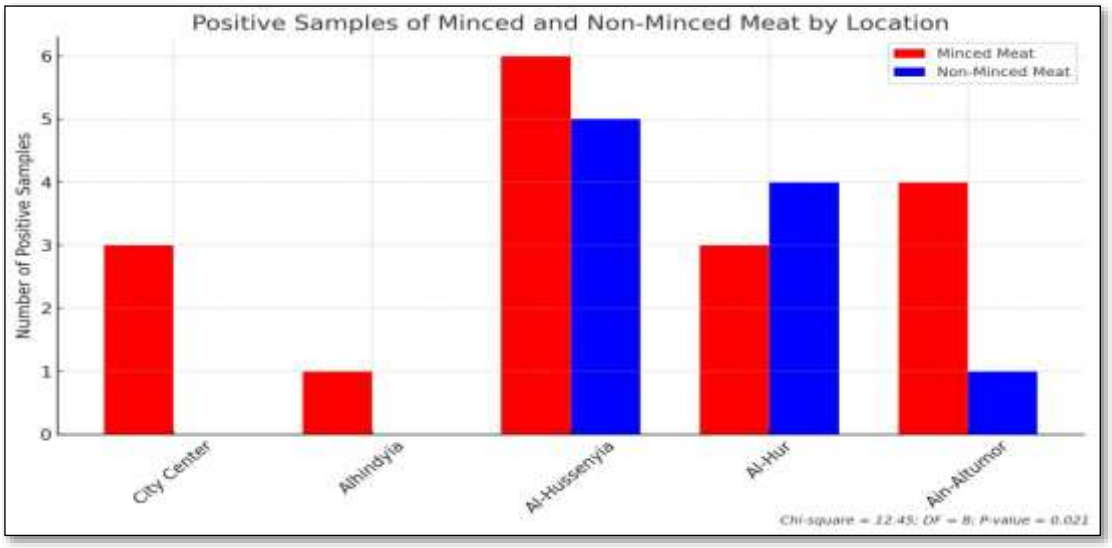


Figure (4-7): isolation of *Pseudomonas Aeroginosa* from meat product collected from different regions

The isolation results showed that the highest percentage of *Pseudomonas aeruginosa* contamination was recorded in raw milk samples, reaching 41.6% of the total samples, reflecting the high propensity of this type of bacteria to multiply in humid environments and unpasteurized animal-based foodstuffs. This high rate is attributed to a lack of hygiene practices during milking or storage, a common problem in rural and semi-urban areas. Similar results were observed in a study by **Ali *et al.* (2023)**, which revealed the presence of *P. aeruginosa* in 38% of raw milk samples in Najaf Governorate. The researchers pointed to the role of unsterilized water and utensils as major sources of contamination. For minced meat, the total isolation rate was 13.6%, with the highest in Al-Hussainiya (24%), followed by Ain Al-Tamr (16%). This reflects variations in the level of implementation of hygiene and meat safety standards between regions, and may indicate problems in cold chains or exposure of meat to air and contamination during processing. These results support the findings of a study by **Hamid *et al.* (2024)**, in Babil

Governorate, where a contamination rate of 19% was recorded in local market meat. The study indicated that *Pseudomonas aeruginosa* can grow even at home refrigerator temperatures, increasing the risk of food poisoning in the event of poor cooking.

Lower isolation rates were recorded in unminced meat (8%), which may be due to less manual handling and cutting compared to minced meat, thus reducing the chances of cross-contamination. Furthermore, no bacteria were isolated from butter in any of the regions, which may be attributed to its high fat content and low water activity, which restricts the growth of aerobic bacteria such as *P. aeruginosa*. This is confirmed by **Farooq *et al.* (2023)**, who reported no growth of *P. aeruginosa* in commercial butter products stored at low temperatures in Basra.

As for local cheese, a total contamination rate of 20% was recorded, and it was higher in the Al-Hur and Ain Al-Tamr regions (32%), which may reflect the use of raw milk in preparation, or storage at inappropriate temperatures. Unpasteurized dairy products are a suitable environment for the growth of *pseudomona*s, especially in the absence of adequate heat treatment. **Najim *et al.* (2024)**, in their study on Kurdish cheeses in Karbala indicated the presence of *P. aeruginosa* in 26% of samples, confirming that this is linked to traditional production techniques and the lack of supervision.

A comparison of locations showed that Al-Hur (21.6%) and Ain Al-Tamr (17.6%) recorded the highest overall contamination rates, which may be related to environmental factors or poor health control in open food markets. In contrast, the Old City (11.2%) had the relatively lowest rates, despite its population density, suggesting the possibility of better health control or greater awareness in food handling. These data are recommended for use in guiding local food control programs, with a focus on areas with the highest rates.

Milk is a nutrient-rich medium, containing sugars (lactose), proteins (casein), fats, and minerals, in addition to a high moisture content and water activity (aW), all of which make it a near-ideal medium for the growth of *Pseudomonasaeruginosa*.Thisbacterium,anaerobic,non-fermentinorganism, has a high capacity to utilize the sugars and amino acids in milk, even at low temperatures (minor growth can occur even at 4°C), which contributes to its rapid spread in raw, unpasteurized milk. Studies such as **Brown & Foster. (2019)**, have shown that *Pseudomonas aeruginosa* prefers moist, nutritious environments and exhibits the ability to secrete enzymes such as proteases and lipases, which degrade milk components and aid its multiplication. Lack of pasteurization or improper storage after milking are also critical factors that promote contamination. Unlike products such as butter or cheese, raw milk undergoes repeated manual handling, whether during milking, transportation, or packaging. This increases the risk of contamination from unsterilized equipment or contaminated water. Furthermore, milk is often not consumed immediately after production, but rather stored for a period that allows bacteria to grow if not refrigerated. A study by **Hassan *et al.***

(2024), indicated that more than 60% of milk samples in southern Iraqi markets contained pathogenic microorganisms, with *Pseudomonas aeruginosa* being the most common, due to poor hygiene measures.

4.4.2. Bacterial Count

A total of $203X10^6$ CFU/ml bacterial colonies were obtained from serial dilution tube number $1X10^6$ CFU/ml. These colonies were confirmed and used to prepare the inoculum strain. This inoculum was later used to induce contamination in meat samples to assess whether meat, or macerated meat, can serve as a suitable growth medium for *Pseudomonas aeruginosa*. Prior to contamination, it was essential to establish the inoculum, which was derived from tube number 6 of the serial dilution series, corresponding to approximately 10^6 CFU/mL, yielding 203 colonies on the agar plate.

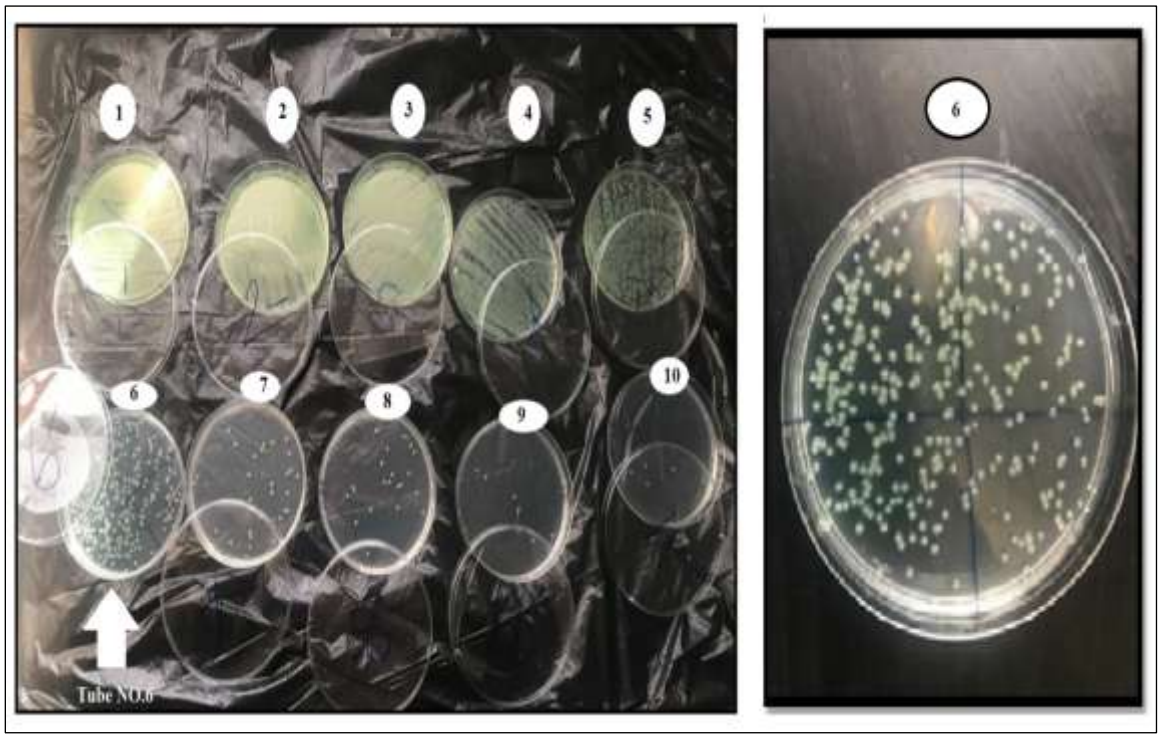


Figure (4-8): Inoculum strain of *Pseudomonas aerogenoza*

4.5. Effect of Temperature and Salts on Bacterial Growth

4.5.1. Effect of Temperature on Bacterial Growth

tables 4-4and 4-5 In milk, cheese, butter, meat and macerated meat, temperature effects on the growth of *Pseudomonas aeruginosa* were studied After 3 and 7 d of incubation at 7-, 21-, 37- and 45-degrees C. After 3 d incubation, temperature had a great impact on the growth. Minimal bacterial proliferation was achieved at 7°C, and the milk, cheese, meat and macerated meat assay presented low CFU values, and no growth found in butter.

Between 21 and 12 °C a slight to heavy growth was observed on all products, with the highest counts recorded on all type products except for butter, where the bacterial counts increased faster especially on milk and cheese. Proliferation was the most intense at 37°C, the temperature was the most favorable for growth of *P. aeruginosa*, in all of the food matrices, except for butter where the most intense multiplication of the tested microorganism was stated at the temperature of 6°C.

There was no growth at 45°C in all sample, which indicated that the microorganism could not survive against higher thermal stress. Presence of bacteria was reduced markedly after 7 days of incubation at sub optimal temperatures. No proliferation was observed at 7°C in any of the products, including those in which only minimal growth had been observed after 3 days. In milk and cheese there was still some growth evident at 21°C (Table 4-8); bacterial survival was not observed at 21°C in meat and similarly with macerated meat, and it is likely that there was diminished viability at extended incubation at this temperature. 37°C (except for butter) was that it was ensuring high bacterial count in all the gradients, proving that this temperature allows bacterial activity to be carried out throughout the experiment. Again, no growth at 45°C and hence thermal inhibition was observed. In each case, no butter at either time point supported any detectable bacterial growth at any temperature, whereas the other products showed temperature-dependent responses of bacteria. These observations suggest that *Pseudomonas aeruginosa* is extremely temperature-sensitive, with optimal growth at 37°C and no growth at 45°C, it thus is likely that

temperature plays a key role in the extent of bacterial survival and proliferation, in both bakging and dairy product as shown in figure (4-8).

Table (4-4) Effect of Temperature on bacterial growth in three days

Colony / Product	Temperature				
	7 C	21 C	37 C	45 C	P value
	$1X10^6$ CFU	$1X10^6$ CFU	$1X10^6$ CFU	$1X10^6$ CFU	
Milk	5 C	307 B	Heavy (1000) A	0 (no growth) D	0.0044
Cheese	15 C	192 B	1000 A	0 (no growth) D	0.0063
Butter	0 (no growth) A	0 (no growth) A	0 (no growth) A	0 (no growth) A	0
Non minced Meat	12 C	58 B	Heavy (1000) A	0 (no growth) D	0.0012
minced meat	17 C	77 B	Heavy (1000) A	0 (no growth) D	0.0045

*Different letters mean significant differences; same letters mean non-significant differences as vertical line

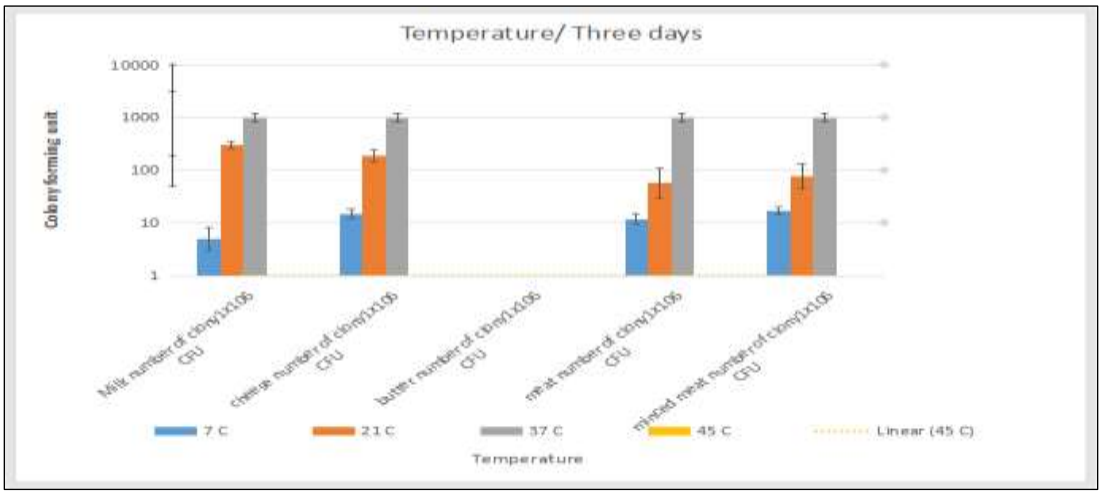


Figure (4-9): Effect of Temperature on bacterial growth in three days

Table (4-5) Effect of Temperature on bacterial growth in Seven days

Colony / Product	Temperature				P value
	7 C	21 C	37 C	45 C	
	$1X10^6$ CFU	$1X10^6$ CFU	$1X10^6$ CFU	$1X10^6$ CFU	
Milk	0 (no growth) C	75 B	250 A	0 (no growth) C	0.0033
Cheese	0 (no growth) C	61 B	200 A	0 (no growth) C	0.0043
Butter	0 (no growth)	0 (no growth)	0 (no growth)	0 (no growth)	0
Non mincd Meat	0 (no growth) B	0 (no growth) B	180 A	0 (no growth) B	0.0013
minced meat	0 (no growth) B	0 (no growth) B	155 A	0 (no growth) B	0.0024

*Different letters mean significant differences; same letters mean non-significant differences as vertical line

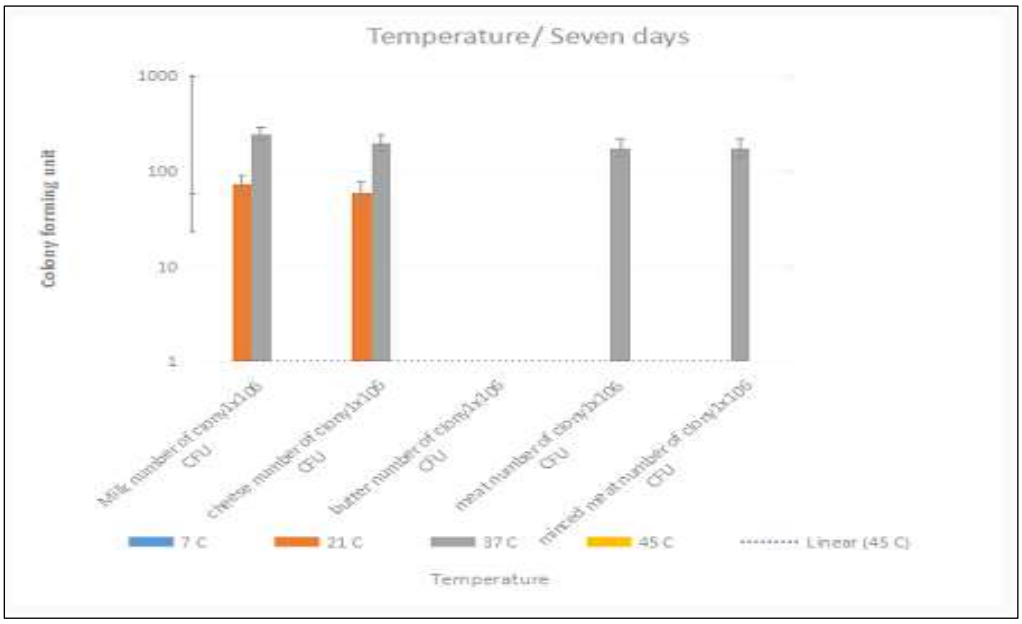


Figure (4-10) Effect of Temperature on bacterial growth in Seven days

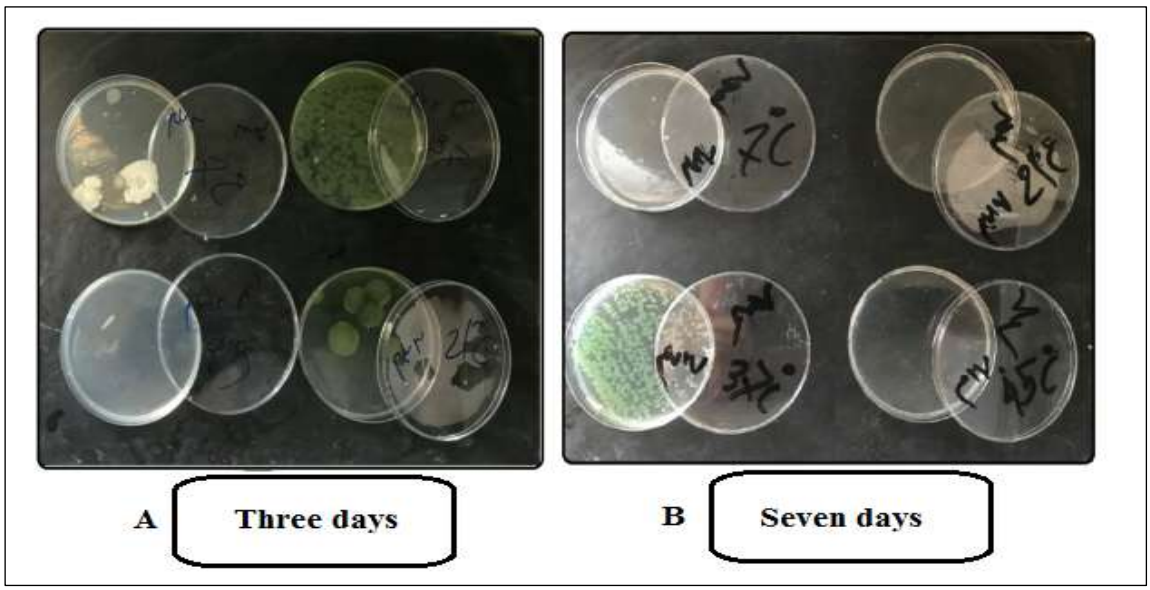


Figure (4-11): Growth of *Pseudomonas aeruginosa* in meat under varying temperature after 3 and 7 days of incubation.

A significant effect of incubation temperature on bacterial growth over a three-day period was observed across the various dairy and meat products, as indicated by the low P values. Bacterial growth, measured in colony forming units (CFU), was found to be highly dependent on the specific temperature condition. For milk, cheese, non-minced meat, and minced meat, the heaviest growth, quantified as 1000 (1x10^6 CFU), was consistently observed at 21°C. This temperature was determined to be optimal for proliferation under the tested conditions **(Phillips, J. D., & Griffiths, 1978)**.

In contrast, incubation at 37°C resulted in no growth being detected for these same products, suggesting that this higher temperature was inhibitory or lethal to the inherent microbial flora. Moderate growth was recorded at the lower temperature of 7°C, with counts of 307, 192, 58, and 77 CFU for milk, cheese, non-minced meat, and minced meat, respectively. The variation in counts at 7°C was noted, with minced meat supporting the highest growth and non-minced meat the lowest among the products that showed growth **(Greene & Jezeski, 1954)**.

Butter was distinguished from the other products by a complete absence of growth at all tested temperatures, including 7°C, 21°C, 37°C, and 45°C. This unique result suggests that the composition or processing of butter creates an environment intrinsically hostile to bacterial survival and replication under these experimental conditions **(Kornacki et al., 2001)**.

Furthermore, the elevated temperature of 45°C was also shown to prevent any bacterial growth in all products, confirming that this represents a supra-

optimal temperature that effectively suppresses microbial development. The statistical significance of these temperature-dependent effects was confirmed by the very low P values, which were all below 0.01, providing strong evidence that the differences in growth were not due to random chance. Ultimately, it is concluded that storage temperature is a critical factor determining the microbiological stability of these perishable goods **(Parvin et al., 2016)**. On the other hand**,** A discussion of the bacterial growth after seven days of incubation, as presented in the accompanying table, is provided herein. A pronounced shift in growth patterns was observed compared to the three-day results, with the optimal temperature for proliferation being identified as 37°C for the majority of products. This temperature effect was determined to be statistically significant, as evidenced by P values all below 0.01 **(Mottar, 2020)**.

For milk and cheese, maximum growth was recorded at 37°C, with counts of 250 and 200 (1x10^6 CFU), respectively. This represents a complete reversal from the three-day data, where 37°C had been inhibitory. Moderate growth was noted at 21°C, while no growth was detected at either 7°C or 45°C for these dairy products. A similar but distinct pattern was found for the meat products. Non-minced meat and minced meat exhibited their highest growth at 37°C, with counts of 180 and 155 (1x10^6 CFU), respectively. However, for these products, no growth was observed at either 21°C or 7°C, in contrast to the dairy products which showed growth at 21°C **(Pal et al., 2016)**.

Butter remained consistent with its previous results, as no bacterial growth was observed at any of the four incubation temperatures after seven

days, further supporting the conclusion that its composition prevents spoilage under these conditions **(Nsofor & Frank, 2012)**.The temperature of 45°C continued to demonstrate a complete inhibitory effect across all products, preventing any measurable growth. The extended incubation period was thus shown to significantly alter the microbial dynamics, allowing for substantial proliferation at 37°C that was not present at the three-day mark, while seemingly exhausting nutrients or leading to self-inhibition at the previously permissive temperature of 21°C for the meat products **(Champagne et al., 1994)**. It is concluded that the interaction between incubation time and temperature is a critical factor governing bacterial growth outcomes.

4.5.2. Effect of Sodium Chloride on Bacterial Growth

After a forced infection of *Pseudomonas aeruginosa*, bacterial growth was determined in milk, cheese, butter, meat and macerated meat, after 3 and 7 days of incubation at various NaCl concentrations (2, 5, 7 and 10%) (See tables 4-6 and 4-7).It was noted that 2% salt promoted the heavy bacterium growth in all tested food except butter, which was bacteria-free. In such low-salt conditions, in milk and cheese, the colony counts were > 250 CFU after 3 d, reflecting marked bacterial growth.

The analogous results were observed in meat and macerated meat (with 68 CFU and 103 CFU, respectively), and, as mentioned above, the dairy and the meat matrices supported growth when NaCl was present at 2%. There was a significant decrease in microorganism numbers at 5% NaCl content. Scantly growth was found for milk and cheese and CFU values for them

reached 9 and 10, respectively, while in macerated meat only 5 CFU were registered. Butter or the whole meat had no growth at this concentration. Without exception, *P. aeruginosa* was entirely inhibited when NaCl did not exceed 7% and 10%, meaning that an increased salt concentration successfully inhibited bacterial survival. Resistance: All effects were significant and the greatest restriction was in butter, with no growth at any salt level. The same tendency was also observed on incubation for 7 days. Significant growth remained at 2% NaCl in milk, cheese, and macerated meat, from which CFU counts were 275, 209, and 215, respectively. Bacteria in milk, cheese, and macerated meat, albeit diminished, were still detectable at 5%. Bacterial growth was not observed at 7% and 10% NaCl in any product that supported growth at less than or equal to these concentrations. The long incubation time period verified that, although P. aeruginosa can grow and multiply in low-salt conditions in the long run, P. aeruginosa is extremely vulnerable to the suppression of NaCl 7% or more. The results showed that the NaCl level is a limiting factor of *Pseudomonas aeruginosa* growth in dairy and meat products, reaching a complete inhibition > or = 7% (see Figure 4-7).

Table (4-6) Effect of NaCl on bacterial growth in Three days

Colony / Product	Sodium Chloride				P value
	2% Con.	5% Con.	7% Con.	10% Con.	
	$1X10^6$ CFU	$1X10^6$ CFU	$1X10^6$ CFU	$1x10^6$ CFU	
Milk	Heavy (1000) A	255 B	9 C	0 (No growth) D	0.044
Cheese	Heavy (1000) A	176 B	10 C	0 (Nogrowth) D	0.0096
Butter	0 (no growth) A	0 (no growth) A	0 (no growth) A	0 (No growth) A	0
Non mimced Meat	Heavy (1000) A	68 B	0 C	0 (No growth) C	0.0012
Minced meat	Heavy (1000) A	103 B	5 C	0 (No growth) D	0.021

*Different letters mean significant differences; same letters mean non-significant differences as vertical line

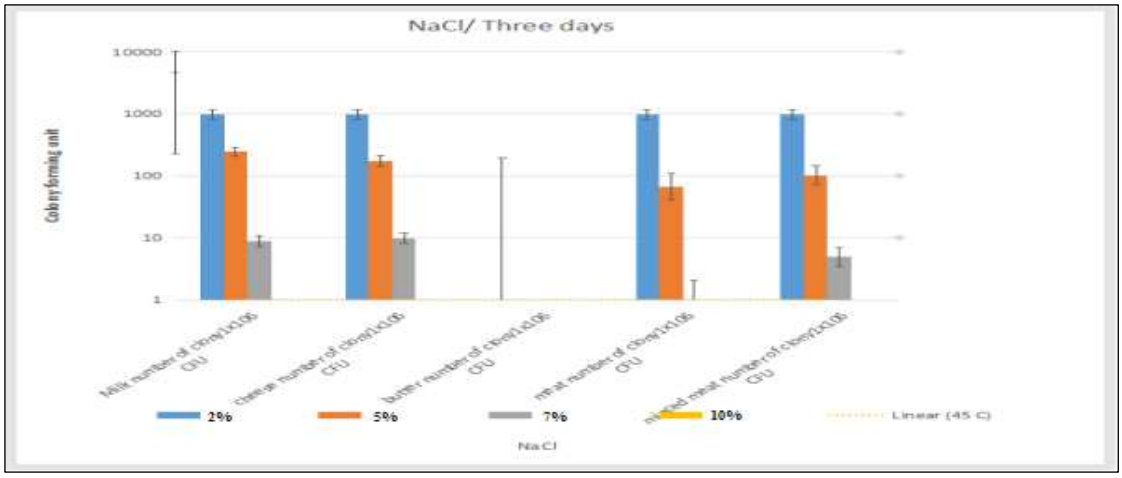


Figure (4-12): Effect of NaCl on bacterial growth in Three days

Table (4-7) Effect of NaCl on bacterial growth in seven days

Colony / Product	Sodium Choloride				P value
	2% Con.	5% Con.	7% Con.	10% Con.	
	$1X10^6$ CFU	$1X10^6$ CFU	$1X10^6$ CFU	$1X10^6$ CFU	
Milk	275 A	50 B	0 (no growth) C	0 (no growth) C	0.0085
Cheese	209 A	23 B	0 (no growth) C	0 (no growth) C	0.083
Butter	0 (no growth) A	0 (no growth) A	0 (no growth) A	0 (no growth) A	0
Non minced meat	180 A	0 (no growth) B	0 (no growth) B	0 (no growth) B	0.0022
Minced meat	215 A	10 B	0 (no growth) C	0 (no growth) C	0.0012

*Different letters mean significant differences; same letters mean non-significant differences avertical line

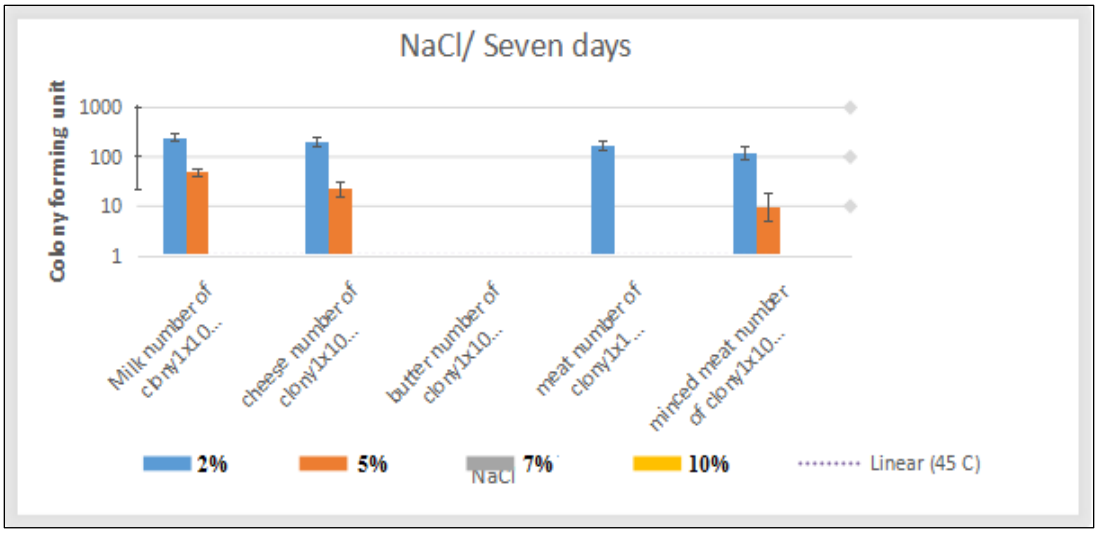


Figure (4-13): Effect of NaCl on bacterial growth in seven days

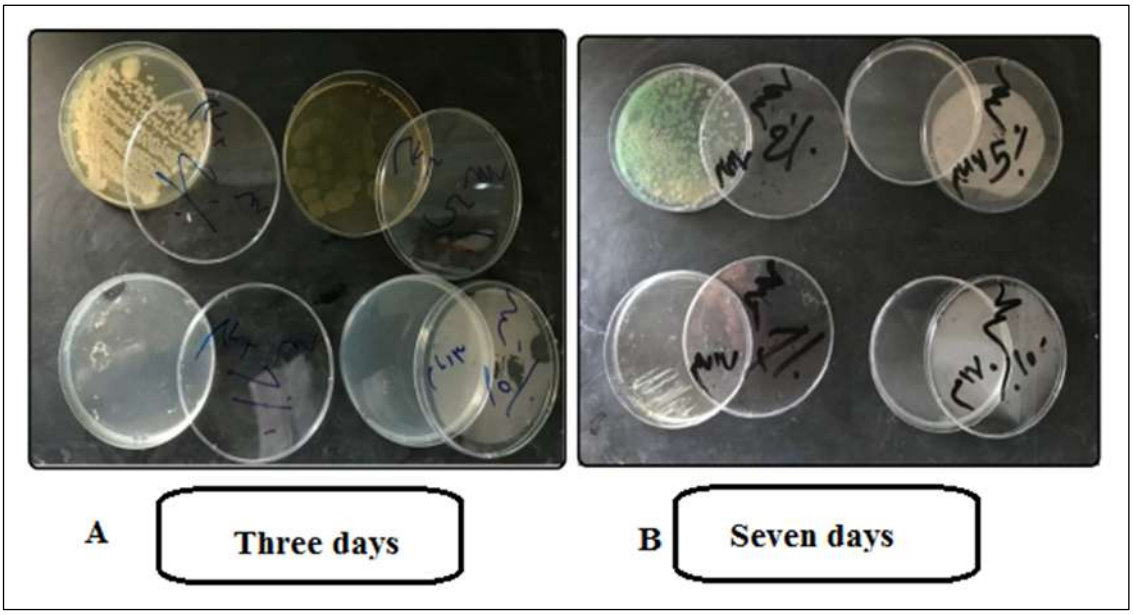


Figure (4-14): Growth of *Pseudomonas aeruginosa* in meat in different concentration of NaCl in three and seven days

The results indicated that *Pseudomonas aeruginosa* has a remarkable ability to grow and multiply in environments with low salt concentrations. Extensive growth was observed in products such as milk, cheese, and minced meat at a salt concentration of 2%. In contrast, a significant decline in growth was observed at 5% salt concentrations, and complete disappearance of growth at 7% and 10% salt concentrations. These results indicate that P. aeruginosa exhibits a clear sensitivity to high salt concentrations, which inhibits its growth. This inhibition is attributed to the osmotic effect, which hinders nutrient absorption and causes cellular dehydration. A recent study demonstrated that adding potassium chloride (KCl) or organic sodium salts such as sodium lactate (NaL) and sodium citrate (NaC) can be effective alternatives to salt in inhibiting the growth of P. aeruginosa in food products. These compounds reduce the water activity and acidify the internal environment of the bacterial cell, thus hindering its growth and multiplication **(Badawy *et al.*, 2023)**.

When analyzing the effect of different salt concentrations on P. aeruginosa growth on days 3 and 5, it was found that low concentrations (2%) allowed for intense bacterial growth, while higher concentrations gradually inhibited growth. This suggests that the bacteria are capable of adapting to moderately salty environments, but are adversely affected by a gradual increase in salt concentration. Another study showed that P. aeruginosa can survive in highly salty environments for extended periods, demonstrating its ability to adapt to harsh environmental conditions **(Cámara-Martos *et al.*, 2015)**.

It is noteworthy to mention that butter showed no bacterial growth at any of the concentrations or days, confirming the unsuitable nature of this fatty substance for the growth of P. aeruginosa. This is attributed to its low water activity and the absence of a moist environment, which is essential for the growth of these aerobic bacteria. A recent study demonstrated that low water activity in food products inhibits the growth of aerobic bacteria such as P. aeruginosa, making butter an unsuitable environment for their proliferation **(Swetha *et al.*, 2017)**. For meat, especially ground meat, intense growth was observed at 2% concentrations, with a significant decrease at 5%, and complete disappearance at 7% and 10%. This reflects a protein-rich environment that is unstable as inhibitory concentrations gradually increase. A recent study demonstrated that P. aeruginosa can continue to grow in refrigerated meats without the addition of effective inhibitors, highlighting the importance of using appropriate salt concentrations or effective preservation techniques to limit the growth of this bacterium.

Statistical P values for most products indicate a significant relationship (<0.05) between concentrations and Pseudomonas growth, underscoring the importance of using inhibitors to control bacterial contamination rates. These results support the recommendation to use inhibitor or salt concentrations ≥7%, or to apply heat/preservative treatments, to ensure that Pseudomonas colonies do not develop in food products. A recent study has shown that the use of salt substitutes such as KCl, NaL, and NaC can be effective in inhibiting the growth of P. aeruginosa, providing multiple options for the food industry to control the growth of this bacteria **(Yadav *et al.*, 2020)**.

Chapter Five

Conclusions

&

Recommendations

6.1. Conclusions

Based on the finding of the present study, the folloing conclusions are obtained

1-*Pseudomonas aeruginosa* is a highly resistant, opportunistic microorganism capable of adapting to a variety of food environments, especially those rich in moisture and protein, such as milk, meat, and cheese. It has demonstrated the ability to grow even in refrigerated or low-temperature conditions, making it a hidden and potentially dangerous source of food contamination.

2- *P. aeruginosa* possesses multiple survival mechanisms, including the production of exoenzymes such as LasB and toxins such as ExoA, which contribute to the breakdown of proteins and lipids and promote its invasion of human tissues, making it a dangerous pathogen.

3-It was noted that the bacteria showed a marked sensitivity to high salt concentrations (7–10%), and highly temperature with their growth significantly reduced under these conditions. This suggests that traditional techniques such as salting or reducing water activity could be used as an effective means of controlling their spread in food products.

4- Unprocessed or improperly stored foods provide an ideal breeding ground for *Pseudomonas aeruginosa*, especially raw milk and ground beef. The findings suggest the need for increased health monitoring of these products to reduce the risk of associated food poisoning.

6.2. Recommendations

The following issues are recommended

1- Strengthening health control over dairy, meat, and cheese products in local markets, especially those displayed at unsuitable temperatures or stored for long periods, to ensure they are free of contamination with *Pseudomonas aeruginosa*

2-The necessity of using salt concentrations 7% or 10% safe alternatives of inhibitory compounds (such as KCl, NaL, NaC) in preserving sensitive products, as they play an effective role in inhibiting the growth of Pseudomonas aeruginosa, as proven by experimental results.

3- using milk hygiene measure during milk process

4- Temperature (45C^{o}) is adaquite for killing the bacteria

5- Conducting periodic bacterial tests in dairy and meat production plants and facilities, using molecular detection techniques (such as PCR) to monitor active genes such as ExoA and LasB, thus preventing the marketing of contaminated products.

6- Isolation and identification of other strains of *Pseudomonas spp*.

Chapter six

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Appendix

Appendix1
AddPrep Bacterial Genomic DNA Extraction Kit

1-A. Lysis protocol for Gram-negative bacteria

1-A-1. Harvest the overnight cultured cell 1 ml ~ 2ml by centrifuge at 13,000 rpm for 30 sec. with 1.5 ml tube (not provided).

1-A-2. Discard the supernatant.

1-A-3 Add 200 μl of Lysis Solution and 20 μl Proteinase K Solution (20 mg/ml) and resuspend the cell pellet by pipetting or vortexing.

1-A-4. Incubate it into 56℃ water bath for 10 minutes. Vortex occasionally during incubation to disperse the sample.

Optional RNase A treatment: If RNA-free genomic DNA is required, add the 20 μl of RNase A Solution (10 mg/ml, not provided).

1-A-5. Add 200 μl of Binding Solution and 200 μl of absolute ethanol and mix well by pulse-vortexing for 15 sec.

1-A-6. Centrifuge at 13,000 rpm for 3 minutes.

1-A-7. Carefully transfer 500 ~ 600 μl of supernatant without pelletinto the upper reservoir of the spin column with 2.0 ml collection

tube without wetting the rim.□ continue with step 2.

Note: If use the procedure of Gram-positive bacterial genomic DNA extraction, the yield of purified DNA of Gram-negative bacteria will

be more 1.5~2.0 foldincreased than lysis protocol of Gram-negative bacteria.

1-B. Lysis protocol for Gram-positive bacteria

1-B-1. Harvest the overnight cultured cell 1 ml ~ 2ml by centrifuge at 13,000 rpm for 30 sec. with 1.5 ml tube (not provided).

1-B-2. Discard the supernatant.

1-B-3. Add 500 µl of Lysozyme Buffer and 20 µl of Lysozyme (50 mg/ml) and resuspend the cell pellet by pipetting or vortexing.

1-B-4. Incubate it into 37℃ water bath for 60 minutes.

Mix welloccasionally during incubation to disperse the sample

1-B-5. Centrifuge at 13,000 rpm for 3 minutes and discard the supernatant.

1-B-6. Add 200 µl of Lysis Solution and 20 µl Proteinase K Solution (20 mg/ml) and resuspend the cell pellet by pipetting or vortexing.

1-B-7. Incubate it into 56℃ water bath for 10 minutes. Vortex occasionally during incubation to disperse the sample.

Optional RNase A treatment: If RNA-free genomic DNA is required, add the 20 µl of RNase A Solution (10 mg/ml, not provided).

1-B-8. Add 200 µl of Binding Solution and 200 µl of absolute ethanol and mix well by pulse-vortexing for 15 sec.

1-B-9. Centrifuge at 13,000 rpm for 3 minutes.

1-B-10. Carefully transfer 500 ~ 600 µl of supernatant without pelletinto the upper reservoir of the spin column with 2.0 ml collection

tube without wetting the rim. continue with step 2.

2. Centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.

3. Add 500 µl of Washing 1 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-

through and assemble the spin column with the 2.0 ml collection tube.

4. Add 500 µl of Washing 2 Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-

through and assemble the spin column with the 2.0 ml collection tube.

5. Dry the spin column by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column.

6. Transfer the spin column to the new 1.5 ml micro-centrifuge tube.

7. Add 100 ~ 200 μl of Elution Solution to the spin column with micro-centrifuge tube, and let stand for at least 1 min.

8. Elute the genomic DNA by centrifugation at 13,000 rpm

Appendix 2

Pseudomonas aeruginosa strain IQ1 16S ribosomal RNA gene, partial sequence

GenBank: PQ836041.1

FASTA Graphics Go to:

```
LOCUS       PQ836041                1130 bp    DNA     linear   BCT 10-JAN-2025
DEFINITION  Pseudomonas aeruginosa strain IQ1 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   PQ836041
VERSION     PQ836041.1
KEYWORDS    .
SOURCE      Pseudomonas aeruginosa
  ORGANISM  Pseudomonas aeruginosa
            Bacteria; Pseudomonadati; Pseudomonadota; Gammaproteobacteria;
            Pseudomonadales; Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (bases 1 to 1130)
  AUTHORS   Bneen,N.H., Kadhim,S.K. and ALi,H.F.
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  JOURNAL   Submitted (05-JAN-2025) Department of microbiology, College of
            veterinary medicine, ghair, iraq, karbala 50061, Iraq
COMMENT     Sequences were screened for chimeras by the submitter using Snap
            Gene Viewer 5.3.2.

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            Sequencing Technology :: Sanger dideoxy sequencing
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```

الخلاصة

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الزائفة الزنجارية (Pseudomonas) هي بكتيريا ممرضة سلبية الغرام، وانتهازية، تنتشر على نطاق واسع في البيئة، بما في ذلك الماء والتربة والمنتجات الغذائية. يُشكل وجودها في الحليب واللحوم مخاطر صحية عامة كبيرة نظرًا لقدرتها على التسبب في عدوى شديدة، وخاصة لدى الأشخاص الذين يعانون من ضعف المناعة. تُعرف هذه البكتيريا بمقاومتها العالية للمضادات الحيوية والحرارة والمطهرات، مما يجعلها ملوثًا ثابتًا في عمليات تجهيز الأغذية وتخزينها. يمكن أن يحدث تلوث منتجات الألبان واللحوم ببكتيريا الزائفة الزنجارية في مراحل مختلفة، بما في ذلك أثناء الحلب والذبح والتجهيز والتخزين غير السليم. قد يؤدي تناول الطعام الملوث إلى التهابات الجهاز الهضمي، وتسمم الدم، وأمراض جهازية أخرى. علاوة على ذلك، تُنتج بعض السلالات سمومًا وأغشية حيوية مستقرة حراريًا، مما يزيد من تهديدها لسلامة الغذاء. شملت الدراسة جمع 625 عينة موزعة في جميع أنحاء مدينة كربلاء المقدسة، مع أخذ 125 عينة من كل من المناطق التالية: مركز المدينة، عين التمر، الهندية، الحسينية، والحر. وُزعت العينات على النحو التالي: 25 عينة من الحليب الخام، و25 عينة من الكريمة، و25 عينة من الزبدة، و25 عينة من اللحوم، و25 عينة من اللحم المفروم جُمعت من الأسواق المحلية. بعد الحضانة والعزل البكتيري باستخدام أوساط زراعة قياسية ومتخصصة، تم تحديد بكتيريا الزائفة الزنجارية وتأكيدها باستخدام نظام VITEK (وطريقة أخرى، إن وُجدت). بعد ذلك، أُجريت تجربة لاختبار مقاومة البكتيريا للحرارة، والتي تضمنت التعرض لثلاث درجات حرارة مختلفة - 7 درجات مئوية، و21 درجة مئوية، و37 درجة مئويةو45درجة - لفترات 3 أيام و7 أيام. بالإضافة إلى ذلك، عُرضت البكتيريا لتركيزات متفاوتة من الأملاح (2%، 5%،7%و10%) لنفس الفترات. زُرعت جميع العينات في أجار الدم، وحُضنت عند درجة حرارة 37 درجة مئوية لمدة 24 ساعة. أظهرت مستعمرات الزائفة الزنجارية المعزولة ما يلي: مستعمرات دائرية، مخاطية، ناعمة، مصابة بانحلال الدم بيتا، مستعمرات صفراء-خضراء في أجار السيتريميد، مستعمرات مسطحة عديمة اللون في أجار ماكونكي، تنبعث منها رائحة تشبه رائحة العنب، إنتاج صبغة زرقاء-خضراء (بيوسيانين) في أجار كينجز أ. جميع عزلات الزائفة الزنجارية من مصادر مختلفة تم اختبارها. نتائج إيجابية للكاتالاز والأوكسيديز. نتائج أجار الحديد كليجلر (KIA، قلوية مع تغيير (أحمر)، وإنتاج لكبريت الهيدروجين، لا تكوين غاز. اختبار سترات سيمونز إيجابي (استخدمت السترات كمصدر

للكربون)، أحمر الميثيل (MR) وفوجيس-بروسكاور (VP): سلبي. نشاط اليورياز: سلبي في جميع العزلات. خضعت عزلات الزائفة الزنجارية المؤكدة

ظاهريًا لاختبار حساسية مضادات الميكروبات باستخدام نظام VITEK-2 الآلي (BioMérieux، فرنسا) وفقًا لبروتوكول الشركة المصنعة. التعرف الجزيئي على الزائفة الزنجارية عن طريق تفاعل البوليميراز المتسلسل الكشف عن جينات الضراوة عبر جينات exoA وlasB خضعت جميع عزلات الزائفة الزنجارية لتجارب جزيئية تعتمد على تفاعل البوليميراز المتسلسل للكشف عن جينات الضراوة الرئيسية، وكشف تضخيم تفاعل البوليميراز المتسلسل متبوعًا بالرحلان الكهربائي للهلام الأجار عن شريط DNA بطول 347 زوجًا قاعديًا، مما يؤكد وجود جين exoA. الكشف عن جين lasB (الإيلاستاز) وبالمثل، حدد تفاعل البوليميراز المتسلسل والرحلان الكهربائي شريط DNA بطول 226 زوجًا قاعديًا، مما يؤكد وجود جين lasB، وتؤكد هذه النتائج التعريف الجزيئي للزائفة الزنجارية في العزلات المختبرة، مما يدعم إمكاناتها المسببة للأمراض بسبب وجود عوامل الضراوة الحرجة. يُلخص هذا التقرير انتشار بكتيريا الزائفة الزنجارية (Pseudomonas aeruginosa) في منتجات الألبان واللحوم المُجمّعة من خمس مناطق مختلفة: مركز المدينة، والهندية، والحسينية، والحر، وعين التمر. في كل موقع، فُحصت 25 عينة من خمس فئات: الحليب الخام، واللحم المفروم، واللحم غير المفروم، والزبدة، والجبن. بلغ إجمالي عدد العينات المُجمّعة 125 عينة لكل منطقة، و625 عينة إجمالاً. سُجّل أعلى معدل تلوث في الحليب الخام، حيث أظهرت 52 عينة من أصل 125 (41.6%) نتائج إيجابية لبكتيريا الزائفة الزنجارية. أما في المنتجات الأخرى، فكان معدل الانتشار أقل: 17 عينة إيجابية (13.6%) من اللحم المفروم، و10 عينات (8%) من اللحم غير المفروم، و25 عينة (20%) من الجبن. والجدير بالذكر أنه لم تظهر أي من عينات الزبدة أي تلوث في جميع المناطق. وعلى الصعيد الإقليمي، سجلت منطقة الحر أعلى عدد من الحالات الإيجابية (23 من أصل 125؛ 21.6٪)، تليها عن كثب منطقة مركز المدينة وعين التمر (22 حالة إيجابية لكل منهما، 17.6٪). وأظهرت منطقتا الحسينية والهندية مستويات تلوث أقل قليلاً، حيث سجلت 19 عينة إيجابية (15.2٪) و18 عينة إيجابية (14.4٪) على التوالي. بينما أظهرت نتائج الاختبارات الفيزيائية أن 37 درجة مئوية هي درجة الحرارة المثلى لنمو البكتيريا ونمو مقيد عند 7 درجات مئوية و21 درجة مئوية أثناء الحضانة المبكرة تليها قمع كامل بعد 7 أيام، فقد وجد أن درجة الحرارة لها تأثير كبير على بقاء البكتيريا وعند درجة حرارة 45 درجة مئوية، لم يُلاحظ أي نمو للبكتيريا.

أظهرت جميع العينات نموًا بكتيريًا ملحوظًا فقط عند تعرضها لتركيز ملح ٪ كلوريد الصوديوم، بينما أظهرت جميع تركيزات كلوريد الصوديوم 5٪ نموًا بكتيريًا ضئيلًا جدًا. عند تركيزات ملح 7% و10

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء /كلية الطب البيطري

فرع الصحه العامه البيطريه

دراسة تاثير تركيز الملح ودرجة الحرارة على حيوية جرثومة الزائفة الزنجارية المعزولة من منتجات الحليب واالحوم في محافظة كربلاء

رسالة مقدمة إلى مجلس كلية الطب البيطري جامعة كربلاء كجزء من متطلبات درجة الماجستير في الطب البيطري / الصحه العامه البيطرية

اعداد

بنين ناجي حسن

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أ.د. **كاظم صالح كاظم**

أ. م. د. **علي حسين فاضل**

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