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Isolation and Identification of Pathogenic Bacteria with Potential for Spoilage from Some Refrigerated Foods after Heating

Research Project
Submitted to the department of (Biology) in partial
fulfillment of the requirements for the degree of B.A or BSc.in biology

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Supervisor Certification

We certify that this research project was prepared under our supervision in the Department of Biology, College of Education, Salahaddin University-Erbil and hereby recommend it to be accepted in partial fulfilment of the requirements for the degree of BSc.

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In view of the available recommendation, I forward this research project for debate by the examining committee.

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Contents

Abstract

Food is a chemically complex matrix, and predicting whether, or how fast, microorganisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in foods; the most important are water availability, pH, and temperature .

Low-temperature storage is a common way to preserve foods that contain heat-stable nutrients. Improper handling with improper use and abuse of temperature while storing these foods may lead to the growth of bacteria initially present in the raw materials. The production of extracellular enzymes by semi-organisms at different temperatures indicates the possibility of their exocytosis.

In this study, thirty samples were collected including rice, red meat, shishkin meat, kofta, doksawa, parma, rice ,Rice with tamato, dagua, green beans, brian, potatoes in water, lentils, Eggs in Tamato, Bulgur and Sarobi samples were collected during the period from the 15th of the October 2022 to the November of the 2022 from the refrigerator after reheating. The samples were cultured on selective and differential media. Several common pathogenic bacterial species were screened by analyzing their morphological and cultural characteristics. *Klebsiella spp* (57.4) was prevalent in most of the samples, while *Entrobactar spp* (4.7) was less prevalent. A large number of samples showed adequate microbial loads at infection or sepsis levels.

Key words :Food spoilage , *Klebsiella spp* , Pathogenic Bactria , Refrigerated

1.Introduction

Isolation and identification of pathogenic bacteria is an important step in identifying the cause of disease in food products (food spoilage) and medical samples to find treatments for them,((Bryan and Organization, 1992, Hill and Wachsmuth, 1996) Food storage is a daily practice in most households. Many of the foods prepared for eating are stored in the refrigerator .(Hebrok and Heidenstrøm, 2019, Marshall, 2021)Refrigeration is the most widely used method to prevent foods for some time. Mostly the perishable foods are kept for refrigeration to control the microbial contamination at 4-50C. (Garden-Robinson.2013) Refrigeration not only minimizes the contamination of foods but also the chemical and enzymatic spoilage of food by retarding the growth of microbes (Scott.*et al.*1982).Storage at low temperature is a popular method for preservation of foods containing heat labile nutrients including vitamins. But improper handling and temperature abuse during transit and storage of these foods may favour growth of microflora initially present in the raw materials (Zhang *et al.*, 2022c). Often we apply heating process to foods may have harmful actions due to possible contamination of foods with harmful microorganisms and reducing the nutritional value of foods (Redmond and Griffith, 2009, Abdulmumeen *et al.*, 2012) Most microorganisms such as fungi and bacteria can cause food spoilage((Zhang *et al.*, 2022a)) .Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics (Abdel-Aziz *et al.*, 2016) . food spoilage bacteria have been considered as the primary causes of food -borne diseases and food quality deterioration in both developed and undeveloping countries(1(Modi *et al.*, 2021) pathogenic bacteria are responsible for producing toxins in food products contaminated with these microorganisms, which pose a high risk for human health. Endotoxins are a class of toxins that are expressed on the outer surface of gram-negative bacteria, while exotoxins are extracellular toxins secreted by bacteria to the surrounding, which induces varieties of host responses and is the primary cause of the

most foodborne diseases(Malekzad *et al.*, 2017)According to data released by WHO in 2020, an estimated 600 million people (nearly one-tenth of the world's people) get sick from eating contaminated food, and 420,000 people die every year, resulting in the loss of 33 million healthy life years (DALYs).(Zhang *et al.*, 2022b). Traditional microbiologists use the following terms to indicate the general (slightly arbi- trary) optimum temperature for growth of bacteria: psychrophiles (15 to 20°C), meso- philes (30 to 37°C), thermophiles (50 to 60°C), and extreme thermophiles (up to 122°C). However, there is a big difference between optimum growth temperatures and the lowest temperatures at which bacteria can grow. Psychrotrophic bacteria can grow at temperatures as low as 0 to 15°C but may be mesophilic (mesophiles) when it comes to their optimum growth range. Psychrotrophs are very important spoilage bacteria in dairy foods under refrigeration. Yeast and molds are common psychrotrophic microorganisms that can cause spoilage of fermented dairy products because of their ability to grow at refrigeration temperatures (4 to 8°C) and under the acidic conditions of these products(Johnson, 2014). domestic refrigerators may be contaminated when introduce contaminated foods, leaking packages, hands, and surfaces which may attach to the internal surface of the refrigerator leading to indirect longer term contamination during activities of subsequent food preparation (Macias-Rodriguez *et al.*, 2013).The bacteria that belong to family of Enterobacteriaceae are considered as more being challenged in the case of being infected to the row and the products of the meat globally. The Salmonella, E. coli , Proteus, and Klebsiella species are the most common species that cause food and some meat products poisoning.(Tilahun *et al.*, 2021)

The aim of the study is to identify and isolate pathogenic food from those contaminated foods Rice ,Read Meat ,Dolma ,Kurdish Pilau, kofta,also In this research we want to aware people more about preparing food and protecting in refrigerator, because food are the source of energy and life, deformed food because of microorganisms especially bacteria cause a big damage of health and economy.

2.Materiale and Methods

Thirty different food samples were obtained after storing them in the refrigerator for two to three days after heating, and then storing them in the refrigerator. The swabs were first dissolved in sterile peptone water.For all samples, after adding 1 gram or 1 milliliter of sample (depending on the type of sample, 1 gram of solid sample with 90 mL of sterile physiological solution or 1 mL of aqueous sample) was added to 4 mL of agar broth, the mixture was homogenized by using shaking and then inoculated on different culture mediaEosin methylene blue agar:, mannitol salt agar, Salmonella-Shigella agar and blood agar. Samples were incubated(at 37 °C for 24 h) and colony growth monitored. Colonies were identified by colony characters and microscopy (Gram staining), (Jose et al., 2015).

3.Results

Thirty different samples were collected from various foods that were cooled after heating and kept for three days after isolation and identification. The result showed that Klebsiella spp was the most prevalent in refrigerated food samples with (57.4%) recorded, and Enterobactar spp (4.7%) as shown in Table (1-3) and Figure (1-3) shows that the percentage of bacterial contamination reached 70% , including 21 samples, and the percentage of non-contamination reached 30%, including 9 samples.

Bacteria	**Gram stain**	**Frequency of occurrence**	**%**
E. coli	Negative	3	14.2
Klebsiella spp	Negative	12	57.4
Proteus.spp	Negative	3	14.3
Pseudomonas.spp	Negaitve	2	9.4
Entrobactar spp	Negaitve	1	4.7

Table(1-3) The percentage of bacterial contamination and non-contamination of refrigerated food after heating it

Figure *1 E.coli* on EMB Agar

This table shows the time the food was stored in the refrigerator, together with Table 3 which shows the growth of bacteria in the food we kept in the refrigerator

Two day from refregrate	**Three day from refregrate**
Doxawa	Rice
Briane	Read meat
Grit	Chichken meat
Chckpea	Kofta
Patato in water	Brma
Fried eggplant	Rice with Mungbean
Kibbeh	Doghawa
Sarupe	Green Bean
Rice with Tamato	Briane
Roasted Chiken Chest	Lentil
Bean	Milk
Kibbeh	Mushroom Soup
Egg in Tamato	Bulgur
Fish Meat	Lemo
Dolma	Shawrma

Table (2-3)The time food are storge from refregrate

Thirty different refrigerated food samples collected from different sources after isolating and identification the result showed that among 30 total samples the growth

ratio is 70 % including 21 samples and the no growth ratio is 30 % including 9 samples as shown in Table(3-3)

	%Growth	**% No Growth**
Rice	+	
Read meat	+	
Chichken meat	+	
Kofta	+	
Doxawa		+
Brma	+	
Rice with Mungbean	+	
Rice with Tamato		+
Doghawa	+	
Green bean		+
Biryane	+	
Patato in water		+
Lentil	+	
Roasted chicken chest	+	
Chickpea		+
Grit		+
Milk	+	
Bean	+	
Dolma	+	
Shawrma	+	
Squash	+	
Fried eggplant		+
Lemo		
Fish meat	+	
Mushroom Soup	+	
Kibbeh		+
Kurdish Pilau	+	
Egg in Tamato	+	
Bulgur	+	
Sarupe		+
Total	21(70%)	9(30%)

Table(3-3) represents bacterial growth and non-growth of refrigerated food after heating

4.Discussion

Food spoiling bacteria are threats to food quality and etiological agents for several pathologies - some debilitating and some fatal.(Kumariya *et al.*, 2019)

Our study displayed that *Klebsiella* was the most frequently isolated bacteria [%57, 4] (Table 1-3), *Klebsiella* ssp. bacteria are normally found in the human intestine. *Klebsiella* ssp. pathogenic strain responsible for multiple nosocomial infections, including pneumonia, urinary tract, and soft tissue infections, (Podschun and Ullmann, 1998). While (Uddin, M.A., Motazzim-ul-Haque, H.M. and Noor, R., 2011) study displayed that E.coli was the most frequent isolated bacteria. And according to the (Taye, Y., Degu, T., Fesseha, H. and Mathewos, M., 2021) study that shows that Lactobacillus spp. is the most frequent isolated bacteria {24.38%}.

According to our study *Entrobactar* spp was the least frequently isolated bacteria (4.7%) {Table 1-3}. However, according to the (El Seify, A.H., Samaha, I.A., Nossair, M.A. and AL-Lami, S.A., 2023) study the *Salmonellae* bacteria was the least frequent isolated bacteria from contaminated foods (6, 0, 0%). And (Ajobiewe, H.F., Bakare, B., Umeji, G.C., Ogundeji, A.A., Udefuna, P.A., Salami, A.O., Aniakor, G.C., Alau, K.K., Yashim, N.A. and Ajobiewe, J.O., 2022) study showed that the *salmonella typhi* had the highest frequency among the isolated bacteria (37.50%).

Our study showed that among 30 samples of in 21 samples positive growth ratio has been found (70%) and in 9 samples negative growth ratio has been found (30%) {Figure (2-3)}. However, according to the (Al-Mutairi, M.F., 2011) study showed that among 75 samples in 15 samples positive growth ration has been found (20%) and in 60 samples negative growth ratio has been found (80%).

Our study displays that among 30 contaminated food samples 5 types of the gram negative bacteria (*E. coli, Klebsiella spp, Entrobactar spp, Proteus.spp, Pseudomonas*.spp) are isolated and identified {table (1-3)}. Vice versa, the (Taye, Y., Degu, T., Fesseha, H. and Mathewos, M., 2021) study displayed that among 3 contaminated food samples 6 types of gram positive bacteria *(Lactobacillus spp.,*

Lactococcus spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Bifidobacteria* spp.) are isolated and identified.

This result of this study of this study seems to confirm the findings of the (Koirala, B., Bhattarai, R., Maharjan, R., Maharjan, S. and Shrestha, S., 2021) as result displayed that among 68 bacteria of isolated and identified bacteria from contaminated food all of them were gram negative bacteria as our study shows that among 21 bacteria that isolated and identified from contaminated foods all of them were gram negative. According to the (Koirala, B., Bhattarai, R., Maharjan, R., Maharjan, S. and Shrestha, S., 2021) study the *Proteus* spp. is the most frequent among the gram negative bacteria that isolated and identified from the contaminated foods (32%). However, according to our study the *Proteus* spp. is the second most frequent gram negative bacteria that isolated and identified from the contaminated foods {table (1-3)}. The frequency result of the (Koirala, B., Bhattarai, R., Maharjan, R., Maharjan, S. and Shrestha, S., 2021) study also showed that the *E. coli* bacteria is the third most frequent bacteria from the contaminated food (13%) which seems to confirm the result of this study in which *E. coli* bacteria is also considered as the third most frequent bacteria from the contaminated food (14.2%) {table (1-3)}.

5.Conclusion

This study showed that pathogenic bacteria can live in refrigerated food samples after heating them, and due to the high number of bacteria observed in food samples, they can thus pose a risk to the health of the consumer. Refrigerated foods can become vectors for foodborne pathogens through abuse of temperature and inadequate cleaning of household refrigerators. It is critical for the public to understand that the refrigerator can be an important habitat for foodborne pathogens to persist and spread due to its reliance on refrigeration as a method of food preservation. Care must be taken when eating refrigerated foods, especially after a long period of time.

To prevent contamination in the refrigerator, there are rules and best practices, includingThoroughly wash your hands before handling and preparing food stored in the refrigerator.Keep ready-to-eat and cooked food above raw food in the refrigerator.Place raw poultry, meat and seafood on the bottom shelf of the refrigerator in food-grade, covered containers or sealed plastic bags to prevent juices from dripping onto other foods.Ensure the fridge is fully emptied prior to cleaning and sanitizing, and that spills are thoroughly cleaned when they occur.Store foods in properly labelled, sanitized containers with airtight lids or seals,Ensure you and your staff are trained in food safety policies and procedures, including contamination prevention.

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