Ministry of Higher Education

And Scientific Research

Babylon University

College of Science

Dep. Of Biology

Isolation and Identification of pathogenic Bacteria from Food Samples

A research

Submitted to the council of the college of science / university of Babylon in

partial fulfillment of the bachelor's in biology / microbiology

By

Ayat Rasool Eabd Alhusayn

Supervised

Prof .Dr. Eman Mohammed Jarallah

2023 A.D. **1444 A.H**

(بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ)

{ قُلْ هل يسْتَوي الذين يعلمُونَ والذين لا يعلَمُونَ }

سورة الزمر:(٩)

Dedication

To the one who taught me how to stand firmly on the ground

Respected father

To the source of love, altruism and generosigenerosi

Respected mother

To my doctor who helped me write this research ،

prof .Dr. Eman Muhammad Jarallah

To all my advice and support

I present to you the summary of my scientific effort

Acknowledgement

For the most mercy for enabling us to present this project in the best form that we wanted to be, we would like to thank our supervisor of this project, (Prof. Dr.Eman Muhammad Jarallah,) for his valuable help and advice to come out with this project. We thank our faculty and doctors that provided us with all the knowledge. Most of all we are all thankful for our families for their endless love, assistance, support and encouragement. And for our friends for their understanding and support for us to complete this project

Abstract

Food safety is a primary focus of food biology; Bacteria, viruses, and disease-causing toxins produced by microorganisms are all potential food contaminants Nine Samples were collected from canned foods (Jam , yogurt and Cheese) .They were Randomly collected from different supermarkets in Hilla, Iraq When isolating and cultivating the samples on Nutrient Agar , the result was, after incubating them, the appearance of bacterial colonies , and in some samples, After cultivating and incubating the samples found that most of the samples from canned food exceeded the number allowed by the biological standards for food safety, A gram stain was performed on the samples, and the samples stained with the Gram formula were seen under the microscope. They were Gram-positive for Staphylococcus spherical stain. When the bacteria were grown on eosin-menthol blue medium, it was inhibited, because gram-positive bacteria could not grow and multiply and form bacterial colonies on eosin-methylene blue medium because it Specific to gram-negative bacteria, when cultured on mannitol agar medium, and after the incubation period, we notice that staphylococcus bacteria (gram-positive) fermentation of mannitol: because the medium takes on a yellow color.

Chapter one: introduction

1.1_ Introduction

Foodborne diseases constitute a public health and economic and social burden worldwide; The World Health Organization (WHO) has estimated that there are 600,000 foodborne diseases and 420,000 deaths, attributed to 31 microbes (Aguirre Garcia et al.,2022; Husain& Aziz, 2022; Pires et ,al, 2021;Mi,etal.,2021).

Meat may contain bacteria, and antibiotic resistance genes, which are transmitted to humans and cause diseases that are resistant to antibiotics, including the beta-lactam group. Sometimes the meat may be uncontaminated, but it becomes contaminated during barbecue operations, including Staphylococcus aureus bacteria carrying methicillin-resistant genes, which reach the consumer due to lack of attention to hygiene operations (Plaza- Rodríguez et al.,2021).

In 2017, the World Health Organization (WHO) recorded twelve types of bacteria that threaten human life, because they are resistant to antibiotics, Bacteria were categorized by priority: critical, high, and medium priority. Pseudomonas aeruginosa was among the critical priority group, Enterococcus faecium, and Staphylococcus aureus, Campylobacter and Salmonella are on the list of high priority group. These bacteria cause symptoms including: fever, vomiting, diarrhea, and stomach cramps (Qi, 2022; Beier, 2021).

1.2_ Review of references

1.2.1 Some types of food are packaged in metal containers and tightly closed, then treated with heat to kill Germs to prevent spoilage, and heat is an important factor for food preservation. (7) The use of high heat It means the

elimination of all microorganisms (cells and spores) with the destruction of the enzymes present in Food, which causes the process of self-decomposition of food tissue, it was found that the use of heat and periods of time .It takes a long time to obtain canned food that is free of germs, but the process is accompanied by some drawbacks.Including the effect on the texture of canned food, changing its color and acquiring an undesirable taste, in addition to the cost. The economic requirements required by the length of the sterilization period, especially in acidic foods. Low (pH > 5.3) and knowing the thermal death time (which is The time required at a given temperature to eliminate certain types of vegetative cells of bacteria or Their boards under certain conditions) has enabled workers in the canning industries to reduce the time period Long periods of heat sterilization (3) and some types of food may be sensitive to heat treatment High and long, like some types of meat and fish, so workers in the canning industry resort to Finding another method known as the commercial method of sterilization, which means that canned food may contain There are some types of bacteria on the boards, but they cannot grow in the correct storage conditions.

There is another method used in treating canned food, which is the pasteurization method.Food, including milk, at a specific temperature and time to eliminate microorganisms Morbidity .(10)

1.2.2 Types of microbial spoilage of cans:

A) Themophilic spore forming bacteria:

These bacteria cause:

1- Flat sour spoilage:

One of the bacteria that causes it is Bacillus sterothermophilus. These bacteria are active inside the can and are acids, the most important of which is lactic acid without gas. Therefore, the can remains flat and does not swell. When the can is opened, an acidic smell appears, as in canned vegetables and milk powder, as agglomeration occurs in the milk, and this damage occurs when stored in an atmosphere It is hot in the presence of spores of this bacteria in the food. Dextrose trypton bromocresol purpol agar medium is used to investigate these bacteria. The medium is incubated at (55°C) for a period of (2-5) days.

2-Thermophilic anaerobic spoilage:

It is caused by heat-loving anaerobic bacteria such as Clostridium thermosaccharolyticum, also called gassy spoilage, to produce a large amount of gases.

3-Sulphid spoilage:

It is caused by anaerobic bacteria, Clostridium nigrificans, especially when there is inaccuracy in the use of heat treatment, where the spores of the bacteria remain, and as a result of its growth, H2S gas is formed, which reacts with iron, forming a black precipitate, FeS, which usually occurs in canned vegetables.

B) spore forming bacteria:

These bacteria belong to the genus Bacillus and Clostridium. The former are active in cans that have not been emptied of air well, while Clostridium grows in foods in anaerobic conditions, forming acids and

gases such as Clostridium butricum, or grows in protein foods such as Clostridium putchlanum.

C) Damage by nonspore-forming bacteria, molds and yeasts:

Its presence indicates ill-treatment or post-treatment contamination, such as Thiobacillus, Leuconostic, Streptococcus and Streptococcus bacteria. As for cold-loving bacteria, their source is the cooling water due to holes in the welding of the can. As for yeasts and molds, their presence is limited to canned sugary foods such as molasses, jam and honey.

1.2.3 _Food Microbiology

Food microbes cause changes that may be beneficial or harmful, affecting the type, quantity and extent of use of food. Food from its natural sources contains some microbes, and it is exposed to contamination with many microbes during circulation, so its microbial content increases and microbes grow in it, causing it to spoil. Also, some pathogenic microbes are transmitted through food, causing diseases to the consumer or secreting toxins that cause food poisoning. On the other hand, microbes are used in preparing and processing Some foods, such as bread, dairy products, pickles, and alcoholic beverages. Microbes are also used in preserving green fodder, such as silage, and in the production of microbial protein.

1.2.4_Food contamination

Food is exposed to contamination from many sources that may be natural sources such as fields, air, water, animals, sewage waste orDuring circulation, transportation and manufacturing, and that the types and numbers of microbes present in the food material determine the ability of the food to be preserved, the speed of corruption to which it is exposed, and the type of treatment required to preserve it.

1.2.4.1_Types of microbial toxins

1- Exogenous toxins: excreted outside the microbe, and the poisoning is caused by the presence of the toxin itself in the food, not the microbe, as in the case of botulinum toxin and staphylococcal poisoning.

2- Endogenous toxins: These are formed inside the microbe, and the poisoning occurs as a result of the microbe being consumed alive, i.e. a microbial infection occurs, where the microbe multiplies in the intestine, and after the death of the microbe and the decomposition of its cells, endotoxins are released causing poisoning, as in the case of poisoning with salmonella and streptococcus bacteria. Prevention methods of microbial food poisoning depend on preventing access to Microbes for food or stop their growth if they reach it

1.2.5_Food spoilage

Biological spoilage occurs in food due to food enzymes, microbes, or both. Microbial spoilage is the most important, followed by enzymatic spoilage. Often, the treatments used to preserve food from microbial spoilage also destroy food enzymes .

Foods are divided according to their perishability into three groups:

A) Non-perishable food

Like sugar, grains and flour, they do not spoil unless they are handled carelessly

B)Medium perishable food

Like potatoes and turnips, they stay healthy for a long time if handled carefully

C)Perishable food

It includes most foods such as vegetables, fruits, meat, poultry, fish, milk and eggs, and these foods are quickly exposed to spoilage unless preserved in an appropriate way.

1.2.6_Types of bacterial food poisoning

1-Botulism poisoning

This poisoning is caused by an exogenous toxin secreted by bacteria (Clostridium botulinum) Symptoms appear after 12-36 hours, with an average of 24 hours after eating food containing the toxin Symptoms are headache, dizziness, difficulty in swallowing, speaking, breathing and looking, then paralysis occurs in the respiratory and nervous systems.The death rate from this poisoning is high, exceeding 65%, and death occurs 3-8

days after the onset of symptoms The microbe is highly resistant to heat and its spores can withstand 120C for several minutes.

The toxin is affected by heat, and therefore the prevention of this poisoning comes from the use of sufficient heat when canning and boiling well before eating the food of questionable quality to avoid the destruction of the toxin, for a period of 15 minutes.

2-Staplylococcal food-poisoning

This poisoning is caused by an intestinal exotoxin secreted by strains of bacteria(Staphylococcus aureus)Symptoms appear according to the sensitivity of the infected person after 1-6 hours, with an average of 3 hours, after eating food containing the toxin. At a temperature of less than 100 C, and that the toxin is very resistant to heat and is not destroyed by boiling. Therefore, to prevent this poisoning, the cooked food should be cooled quickly and placed in the refrigerator to stop the growth and activity of the microbe so that the toxin does not form.

3-Salmonella food poisoning

This poisoning is caused by a microbial infection from bacteria

(Salmonella typhimurium) ,(Salmonella entriditis)

Symptoms appear after 7-30 hours, with an average of 24 hours after infection. The length of the incubation period distinguishes salmonella poisoning from staphylococcal poisoning (3 hours on average).

1.2.7_Food preservation

All preserving methods depend on one or more of the following:

1- Keeping away or preventing pollution
2- Inhibiting microbial growth
3- Killing microbes.

The appropriate preservation method determines the type of food and the conditions on which it is found. It is advisable to use more than one method for preserving one food, as there is rarely a single method that is suitable and sufficient in all respects. Preservation

methods are not a substitute for cleanliness, but health conditions must be observed in production and collection.

Chapter two: Materials and Methods

2.1_Materials and methods

Nm	Tools and devices	Materials
1	Beaker	Beef luncheon meat
2	Glass cylinder	Canned cheddar cheese
3	Flat heater device	Canned cream cheese
4	Clean paper	Canned kale cheese
5	Hanging device	Canned carrot cheese
6	Hood device	Canned fig cheese
7	Autoclave device	Canned apricot cheese
8	Incubator	Canned tuna fish
9	Tubes	Chicken luncheon meat
10	Implant dishes	Nutrient agar
11	Syringes	Distilled water
12	Pen	Normal slane
13	Cloves	Crystal Violet
14	lab coat	Iodine and alcohol
15	Microscope	Safranin

2.2 Nutrient Agar

A petri tray containing a culture medium (usually consisting of agar plus nutrients) used to grow microbes or small plants such as the moss Sycometrella betens.

Mukawinat biyat alajar almughadhiy:

1- peptone 6 jum/lun
2- yeast extract 2 jum/l
3- Beef extract 1 jum/l
4- sodium chloride 0.5 jim /l
5- Ajar agar 14 jum/l

Tudhab hadhih almawadu fi litr ma' muqatar

2.2.1Action steps to prepare the nutrient agar environment:

1- We weigh the quantities shown above, taking into account the quantity that we want to prepare.

However, currently some companies have installed this environment in one package, showing the quantity required to prepare one liter of the food environment.

2- This quantity is placed in a clean conical flask.
3- We measure the required amount of distilled water using the graduated cylinder, then add it to the beaker.
4- We put the beaker on the flame or using the water bath for heating, taking into account the continuous stirring so that the environment

does not burn and we continue until boiling. This process is intended to homogenize the materials and ensure that the agar melts well.

5- We prepare test tubes and fill them up to half. The purpose of this is to make slant agar tubes so that the tubes are supported on a slightly elevated surface at an angle of 45° and left to solidify.

Or to make deep agar tubes, which are left vertically in the tube holder until hardened – after being sterilized.

6- We close the beaker and tubes with tight cotton plugs and cover them with tin foil.

7- They are placed in an autoclave for 20 minutes at 2 o C for sterilization, then we take them out and leave them to cool for use or keep them until needed.

2.3 The method of work

Three samples of canned cheese in the markets, three samples of canned meat, and three samples of canned jam were collected from different places in the markets in Hilla. The work was conducted inside the university laboratory under sterilization conditions. We prepare the agar neurite The ready-made from the company, which is in the form of powder, according to the instructions on the box, and this is done by placing a clean, empty sheet of paper for the purpose of zeroing the device, and then we measure the required amount of neutron agar, and then we put 100 grams of water distilled In the beaker for the purpose of measurement, then we put the measured and 2.82 of the neutron Agar in a glass cylinder and stir a little for the purpose of homogenization and

put it on a hot heater for a few seconds, then close the mouth of the flask with cotton and cellophane to prevent water distilled and moisture from entering the inside of the flask when placed in the autoclave with the help of the specialist professor in the laboratory and after 15-20 minutes have passed at a temperature equivalent to 121 degrees Celsius, and after the period ends, the agar is taken out, and we let it cool down until it becomes a warm temperature before use

After that, we whistle the balance device in the same way mentioned above, weigh 0.1 grams of the selected sample, prepare six tubes, and mark on each tube its own number, and put in the

first tube 0.1 grams of the sample and 9 ml of Normal Slane By means of the syringe , mix well using the sample shaker device, and then put in each tube From the tubes 9 ml of Normal Slane By means of the syringe and we transfer 0.1 ml from the first urine to the second tube and 0.1 ml from the second tube to the third tube and so on until we reach the sixth tube and neglect 0.1 ml of it

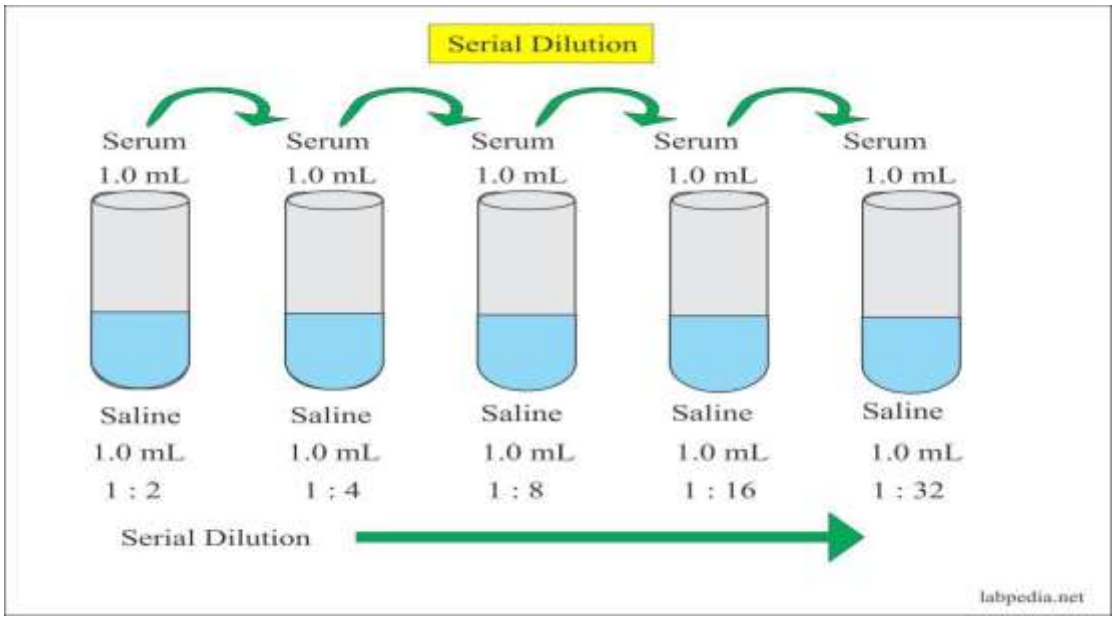

Figure (2.1): dilution method

- After culturing the bacteria, incubating and growing the colonies, a gram stain is performed to diagnose whether the bacteria are gram-negative or positive.

2.4_Gram stain :

The Gram stain is one of the most important types of dyes used in hospitals to identify the type of bacteria, and the attending physician can know the type of bacteria initially to start treating the patient with the appropriate antibiotic quickly. Thanks for its discovery to the doctor of Danish origin, Hans Christian Gram, who was working in the anatomy laboratory of the Berlin

Hospital in the year 1880 AD. Where he developed this method to help him differentiate between the types of bacteria that cause pneumonia, where he noticed that one type of bacteria was dyed red when placed in a specific solution containing iodine and called it (gram-negative bacteria) and another type of bacteria was colored blue, and named it (Gram-positive bacteria). The color of bacteria in a Gram stain depends on the chemical composition of the cell wall. .

2.4.1_Gram stain method

All types of bacteria are stained with Gram stain through several steps in order, and at a specific time, and the bacteria taking the dye depends on the thickness of its cell wall.

Gram-positive bacteria have a thick cell wall; Which makes them acquire the first dye only and never lose it. As for Gram-negative bacteria, their cell wall is thin; Which makes it lose the first dye easily, and gain the second dye, and the steps of dyeing bacteria with the Gram stain include the following:

1- Get a glass test slide and swab it for bacteria.
2- Stabilizing the bacteria on the slide, by passing the slide over the flame several times.
3- Placing the basic dye, Crystal Violet, on the slide for one minute, and the bacteria will acquire a violet color.
4- Wash the slide with water; To remove excess dye from the slide.
5- Then the slide was rinsed with iodine solution; To set the dye,
6- Followed by an organic solvent such as alcohol or acetone

7- Gram-positive bacteria remain purple; Because it has a thick cell wall that is not easily permeable by the solvent; However, Gram-negative bacteria are decolorized; Because they have cell walls with much thinner layers, they allow removal of the dye by the solvent.
8- In a final step, Safranin was added to the slide, which gives Gram-negative cells a red color.

*****After completing the process of staining the bacteria with a gram stain, the dominant was placed under the microscope at and the bacteria were gram-positive in a spherical form (Staphylococcus)

*****After knowing the shape of the bacteria under the microscope, positive bacteria appeared with a spherical Gram formula. The bacteria will be grown on eosin-methylene blue medium**.**

2.5Eosin Methylene Blue:

Media (EMB): stands for (Eosin Methylene Blue) as it is one of the types of media that are prepared industrially within the clinical microbiology laboratory, as it is considered a type of selective and differential medium because it promotes the growth of Gram-negative bacteria and inhibits the growth of Gram-positive bacteria Gram, as Gram-positive bacteria cannot grow, multiply and form bacterial colonies through them.

In addition, it is used to distinguish between types of gram-negative bacteria, because it contains dyes and types of sugar in its components. For example, this media contains lactose in its components. Some types of gram-negative bacteria can ferment lactose, such as (E. coli), and thus give a green color. Bright compared to bacteria (Pseudomonas, Salmonella, Shigella) that cannot ferment lactose.

2.5.1The most Important components of EMB Media:

The media (EMB) is prepared industrially in the laboratory, as during the preparation of the media it is taken into account that it is of the type (selective and differential medium) i.e. it is selective, i.e. it promotes the growth of gram-negative bacteria and inhibits the growth of gram-wave bacteria due to They contain both dyes (Eosin and Methylene blue), where the presence of this type of dye in the composition of the media leads to the inhibition of the growth of gram-positive bacteria, either in relation to it being differential (differential) due to the presence in its composition of lactose ferments that is used to distinguish between types of negative bacteria Gram.

2.5.2 components of this media:

1_ Distilled water

2_ Components of digested animal tissues.

3_ Dipotassium phosphate in very small proportions.

4_ Lactose Components of digested animal tissues.

5_ Dipotassium phosphate in very small proportions.

6_ Lactose

2.5.3 Sucrose EMB media preparation method:

1_ Wear gloves and masks, and sterilize the workspace with chemical disinfectants. Sterilization of all tools and equipment used in the media preparation process.

2_ Put distilled water in the sterilized glass vial in an amount suitable for the amount of media to be made.

3_ Put the glass flask (Flask) on the fire source, put the magnetic stirrer, then add the manufactured agar slowly and gradually before heating the water so that the agar does not clump and dissolves completely.

4_ Continuous monitoring of the liquid until it reaches the boiling point and turns into the pure form.

5_ The glass flask is cooled by placing it in a water bath for 15 to 20 minutes at a temperature of 50°C. The purpose of the water bath is to oxidize (methylene blue) and suspend all the sediment.

6_ Pour the mixture into sterile Petri plates and allow it to come to room temperature.

7_ The date and time of preparation are noted on Petri plates and placed in a sterilized refrigerator at a temperature ranging between 2°C and 6°C.

8_ Sterilization of all tools used in the preparation process, in addition to sterilization of the work area.

*After culturing the bacteria and incubating, we notice that the growth of gram-positive bacteria is inhibited, as gram-positive bacteria cannot grow,

multiply and form bacterial colonies on eosin-methylene blue medium because it is specific to gram-negative bacteria**.**

2.6Mannitol salt agar

Is a medium for the growth of microorganisms.[1][2][3] This medium helps the growth of a certain type of bacteria. This medium is important in medical laboratories to differentiate between pathogenic microbes in a short time. The medium contains a high concentration of salt (7% - 10%), making the medium limiting the growth of Micrococcaceae and Staphylococcus bacteria. Also, it is considered as a differentiator between the types of Staphylococcus bacteria by using mannitol sugar and using a pH limiter (phenol red). Cocoylase-positive bacteria have yellow colonies and other bacteria take a red color. This medium is used to isolate pathogenic Staphylococcus bacteria.

*****And because it is differential because this medium contains a pH indicator to determine the microorganisms that ferment mannitol, as the organic results or organic residues resulting from mannitol fermentation lead to a change in the color of the media from red to brown. One of the most important types of bacteria that grow on this media is the Staphylococcus family

*****The aim of growing bacteria on mannitol salt agar medium was used to distinguish the growth of the Staphylococci family from other families of Gram-positive bacteria.

2.6.1The most Important components that must be available during the preparation of (mannitol salt agar):

1_ Distilled water

2_Nacl

3_Phenol Read

4_Pep_tones

5_Agar

6_Index (PH) pH values should range around 7.2.

7_Digested and fertilized animal tissues.

*****After growing the bacteria on mannitol medium, salt agar, and after incubation, we notice staphylococcal bacteria (gram-positive) fermenting mannitol: because the medium takes on a yellow color.

Antibiotic sensitivity test

Antibiotic susceptibility testing

Quantification of sensitivity:

The sensitivity of microbes to different antibiotics is estimated by the ability of these antibiotics to inhibit the growth of these microbes. There are several methods for estimating antibiotic sensitivity, including qualitative and quantitative methods.

The purpose of conducting a sensitivity test is to find out whether the causative organism is sensitive or insensitive (resistant) to a number of

antibiotics that are closely related to the patient's treatment. Before conducting any sensitivity test, the following must be taken into account:

1- Knowing the genetic background of in vitro microbes, because some types of microbes have mutations.

2- The extent of the sensitivity of the strain under test compared to individuals of the same species.

3- Knowing about the antibody under test, such as its toxicity, composition, absorption by the body, and its mode of action.

Methods for estimating sensitivity:

1) Dilution method

2) Diffusion methods of sensitivity testing.

Disk Diffusion Method

It is the most widely used in veterinary medicine because of its ease of use and low price.

In which bacteria are grown in agar and specific quantities of several antibiotics are added inside it to start spreading. Based on the nature of its spread inside the tablet, the most effective antibiotic is determined.

Diffusion methods of sensitivity testing The disc diffusion method involves selecting a strain of bacteria, placing it on an agar plate (often using Mueller-Hetone agar), and observing the growth of bacteria near the antibiotic-impregnated discs. This is also called the Kirby-Bauer method, although of

using modified methods also. Small paper tablets containing antibiotics are placed on a plate on which bacteria grow. If the antibiotic inhibits microbial growth, a clear ring or zone of inhibition appears around the disc. Bacteria are classified as biosensitive by comparing the diameter of the zone.

2.7_ culture of bacteria:

is prepare two dishes from the culture dishes and mark on each plate the number of the dilution, so we write on the first dish 105 and write on the second dish 10 6 and then we put in the first dish 0.1 ml of Tube No. 5 by means of a syringe and add an appropriate amount of neutron agar and the work is always inside the hood Close to the burner, in order to achieve the sterilization conditions and to obtain correct results, and then add 0.1 m of Tube No. 6 by means of a syringe, add an appropriate amount of neutron agar, put a cover on the Petri dishes after completing the work, and move the two dishes clockwise and sometimes counterclockwise slightly, in order to homogenize The sample with neutron agar and leave the two plates for the neutron agar to solidify

As for when cultivating a sample of kale cheese, the result was the appearance of a few bacterial colonies in the culture dish after leaving the incubator .

This result is somewhat correct because in such types of samples the number of bacteria in them is small due to the use of chemical preservatives in the food industry to improve the quality of the product, but what we know is that most of the existing bacteria have a role that goes beyond giving the delicious taste to cheese, but also has a role in Its

safety and preservation as it secretes inhibitors against other pathogenic bacteria such as Listeria.

After 24 hours, we take the dishes out of the incubator and note the growth of colonies in the dish and count the number of colonies by applying the colony counting law

Chapter Three
Result &Discussion

CFU/ml = NO.of colonies × dilution factor × plating factor

We apply the following law, and if the number of colonies ranges from (30-300), it is and fit for consumption, but if it is more than that number, the sample is Not suitable for consumptio

Table 1.3 Guidance on the interpretation of results for hygiene indicator organisms in ready-to-eat food in general(Centre for Food Safety. (2014)

Hygiene indicator organism	Result (colony-forming unit (cfu)/g)		
	Satisfactory	Borderline	Unsatisfactory
Enterobacteriaceae [a]	$<10^2$	10^2 - $<10^4$	$>10^4$
Escherichia coli [b]	<20	20 - $<10^2$	$> 10^2$

To be implemented when the testing capacity for this criterion is ready.

The figure below shows the bacterial colonies present in the culture dish that contains neutron agar medium after leaving the incubator for a sample of canned carrot jam.

The results of the research were all samples are pathogenic to the consumer and that According to the following table **:**

Table3.2: Shows the number of organisms present in canned food and its impact on the consumer

Nmber Sample	Canned food samples	Agricultural medium	The number of organisms present at a dilution of 1/10^5	The number of organisms present at a dilution of 1/10^6	Interpretation of the results
Sample1	Canned cheddar cheese	Neutriente agar	1.7	1.3	satisafactory
Sample2	Canned cream cheese	Neutriente agar	7.3	5.8	satisafactory
Sample3	Canned kale cheese	Neutriente agar	1	7	Satisafactory.
Sample4	Canned carrot cheese	Neutriente agar	3.5	1	Satisafactory.
Sample5	Canned fig cheese	Neutriente agar	5.28	4.32	satisafactory
Sample6	Canned apricot cheese	Neutriente agar	1.7	1.2	Satisafactory
Sample7	Canned tuna fish	Neutriente agar	1.15	3.5	satisafactory
Sample8	Chicken luncheon meat	Neutriente agar	3.2	1.5	satisafactory
Sample9	Beef luncheon meat	Neutriente agar	7.2	5.12	satisafactory

Table 3-3: Antibiotics senstivity test

Antibiotic	R	S
E	98 %	2 %
DA	98 %	2 %
TMP	100 %	0 %
LEV	0 %	100
C	96 %	4 %
TE	84 0%	14 %
CIP	0 %	100 %

Sensitivity test was performed, which includes the following antibiotics Erythromycin€,Chloram©,Ciprofloxacin(CIP),Trimethoprim(TMP), ,Clindamycin (DA) , ,Tetracycline(TE) and Levofloxacin(LEV) .

Erythromycin Trimethoprim have been shown to be resistant to antibiotics. On the contrary, there are antibiotics that are highly sensitive to antibiotics, such as Levofloxacin and Ciprofloxacin.

Identification of Bacterial Isolates

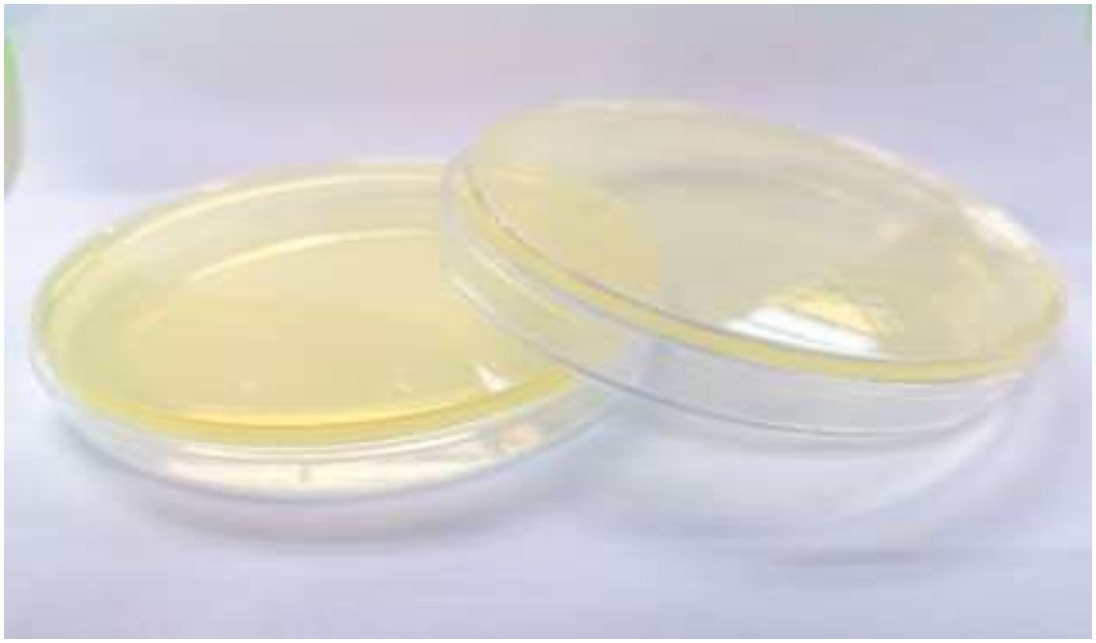

Figure 3.1: Petri dishes illustrating the culture of bacteria

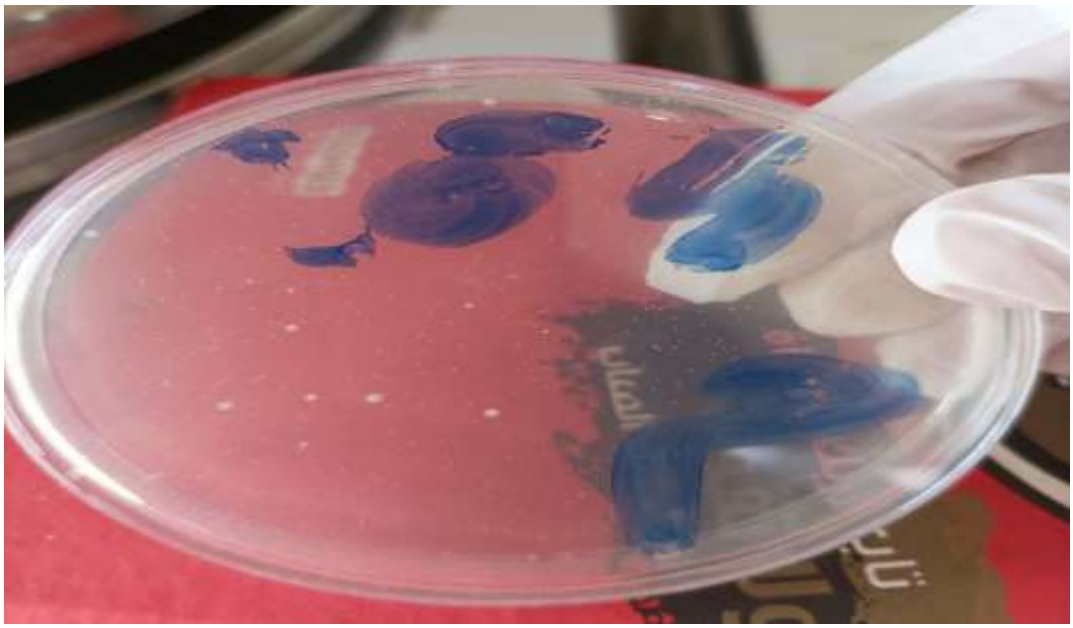

Figure 3.2:Bacterial colonies in a petri dis A)Number colonies from Fifth dilution

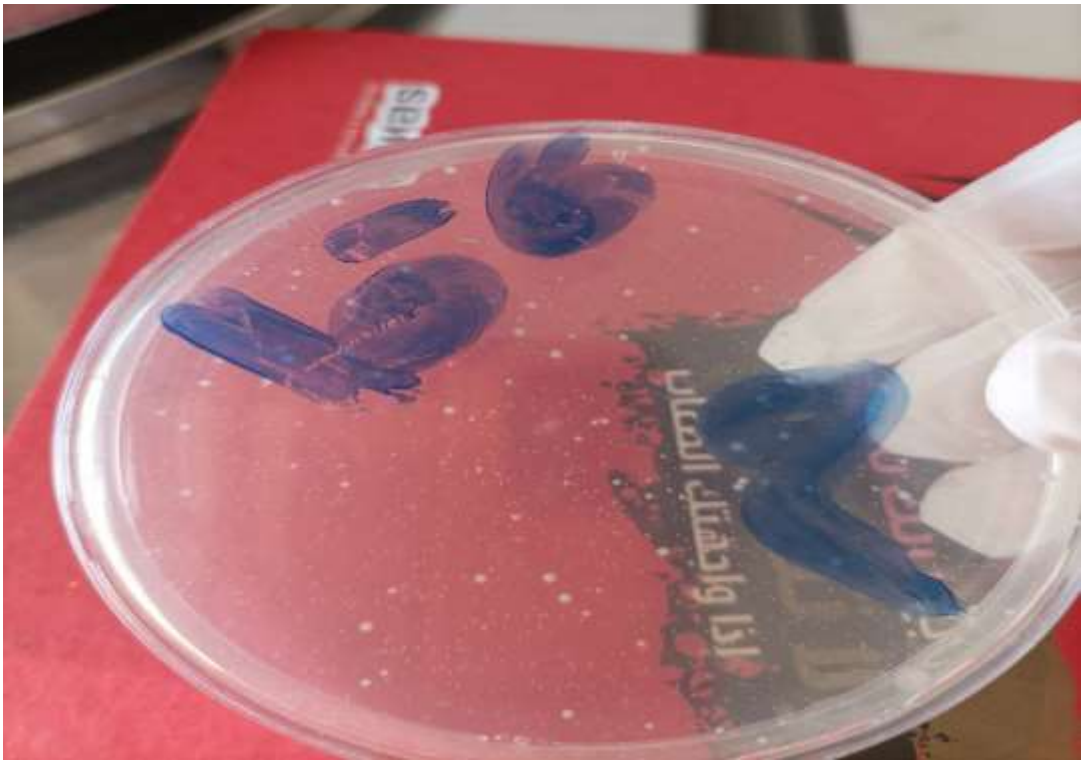

Figure 3.3: Bacterial colonies in a petri dis B)Number colonies fromSixth dilution

Figure 3.4:After completing the process of staining the bacteria with a gram stain, the dominant was placed under the microscope at and the bacteria were gram-positive in a spherical form (Staphylococcus)

Figure3.5:Eosin Methylene Blue:After culturing the bacteria and incubating, we notice that the growth of gram-positive bacteria is inhibited, as gram-positive bacteria cannot grow, multiply and form.

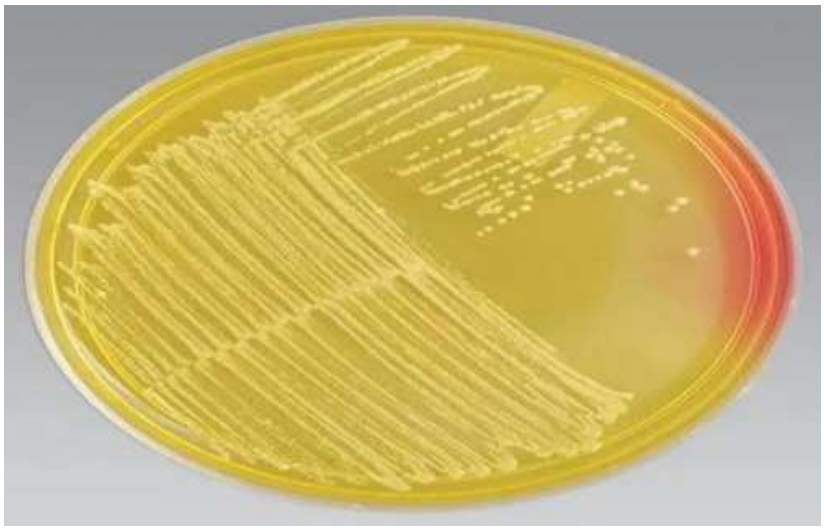

Figure3.6:bacterial colonies on eosin-methylene blue medium because it is specific to gram-negative bacteria

Discussion

The results of this study indicate that food of animal origin can be a source of Antimicrobial resistance of staphylococcus, Enterobacteriaceae, and other Bacteria, which may spread through the food chain, and increase the importance Of these findings for public health. Raw animal meat may be a source of resistant Pathogens that jeopardize the sanitary use of raw meat. However, further study is Needed if more epidemiological characteristics are to be known.Antimicrobial sensitivity testing in the present study has demonstrated multi-drug Resistance((at least one antimicrobial drug in three or more l classes of antibiotics)By calculating the number of bacterial colonies, it was found that most of the samples from canned food exceeded the number allowed by the biological standards for food safety. When counting colonies, however, no more than 30-300 colonies per plate should be used. (Artés et al.,2007; Mekonnen, 2011). This is due to non-compliance with the factors that prevent the growth of Bacteria that cause food spoilage or disease.The highest levels of bacterial contamination were in Tazaj beef luncheon, Esalat chelavra, Chicken luncheon altazaj althabi, Sardines, Silou tuna and Chicken pieces nasma, where the number of colonies exceeded the permissible numbers. Where the majority of canned foods were on the shelves at room temperature, as the temperature is very high in the summer in Iraq, which allows them to grow bacteria at the appropriate temperature. As for frozen meat products, in which the number of bacteria increased, this is due to several reasons, the most important of which is thawing the products and refreezing them again due to the fluctuation of electric current It was observed when meat samples were taken from the butcher Immediately after the slaughtering operations and cultured in the medium, that No bacteria were

isolated in the medium. This is evidence that the meat is free of Pathogens, but contamination arise from slaughtering, transportation and Processing. But when meat from butchers was taken at separate intervals of the day, it was noticed that the numbers of isolated bacteria increased, especially from minced meat intended for grilling during the night.Samples of bacteria growing were taken on Nutrient Agar medium, purified on Nutrient Agar medium again by sub-culturing method, then incubated at 37 °C for 24 hours, then this step was repeated until pure colonies were obtained. Pure colonies were grown on selective media to characterize the isolated bacteria. To ensure the purity of the samples, the samples were subcultured more than four times for each sample. When the plates were subculturing for purification, there were samples evenly distributed in each plate. Samples were also cultured on MacConkey agar and to distinguish.Gram-negative bacteria, Gram-negative enteric bacteria are isolated and Separated into lactose-ferment-positive and lactose-ferment-negative bacteria Using MacConkey agar. This medium's selective effect is owing to the fact that it Comprises: Crystal violet and bile salts inhibit the majority of Gram-positive Bacteria. The medium's osmotic balance is maintained by sodium chloride. Agar Is a hardening agent that becomes red when the pH is less than 6.8 and colorless When the pH is larger than 6.8 MacConkey agar is used to isolate bacteria that Live in Gram-negative intestines. It is also used to distinguish between Gram negative bacteria that are lactose-ferment-positive and Gram-negative bacteria That ferment lactose-negative.It is used to isolate coliforms and intestinal pathogens. Growth of positive Strains that ferment lactose are red or pink. Negative strains that ferment lactose, Such as Salmonella, and Shigella, are colorless and transparent and usually do Not change the appearance of the medium. Bacteria were cultured in the medium of Blood agar is used to

discover cytolytic toxins that cause hemolysis (the destruction of RBCs). Generated by bacteria such Bacillus, Streptococcus, Staphylococcus, Enterococcus, and Aerococcus. Blood Agar is used in this experiment to distinguish Microorganisms with various hemolytic characteristics (Beta-hemolysis (β-hemolysis), Gamma-hemolysis (γ-hemolysis) (or non-hemolytic), and alphahemolysis (α-hemolysis .About 90% of the isolated bacteria were (β-hemolysis) That is, it is a product cytolytic toxins.Microscopic examination shows the arrangement of cells using Gram Stain, which showed different .shapes according to the type of bacteria, where They are divided into two sections, a Gram-positive section stained in violet Color, and a Gram-negative section staining an orange color due to the thickness Of the cell wall of the bacteria. The prevalence of S. aureus was closer to the present study have been reported By Sahin et al. (2020) in Turkey (32%), and Wang et al. (2013) in China (24.2%)Naas et al. (2019) in Libya (32%).Another study that reported by (mathenge et al .,2015) in which an overall of 36% in meat and milk product was found in Nairobi county its surroundings

Conclusions
&
Recommendation

Conclusions

 All canned samples were contaminated with different bacteria, but the most prevalent was staphylococcus aureus.

 Fresh meat samples taken immediately after slaughter were free of any bacteria when grown on nutrient agar medium. While meat samples taken at successive intervals were found to contain bacteria.

 The most important bacteria discovered are of Enterobacteriaceae, Enterobacter cloacae, and Klebsiella pneumonia, Staphylococcus aureus, Gemella sanguinis, and Staphylococcus lentus, and other bacteria Burkholderia cepacia, Kocuria kristinae, Micrococcus luteus, and Streptcoccus agalactiae.

 The highest percentage of enterotoxins genes were detected in S. aureus isolates were as follows : sea, seb, sec and sed, sec which were in equal proportions. While fem A genes were not detected in this study.

 All isolates resist to Trimethoprim (TMP10). And a penicillin G (P), followed by Erythromycin, Clindamycin (AD),chloramphenicol ©, Vancomycin (VA), tetracycline (TE), Oxacillin (OX) , the lowest resistance against Nitrofurantoin (F-100) , rifampicin (RA), and gentamicin (CN), and no isolates found resistant to Nitrofurantoin (F), Ciprofloxacin (CIP), and Levofloxacin (LEV) which makes them a good choice for treatment, and Gram-negative bacteria were more resistant to antibiotics than Gram-positive bacteria.

The highest contamination of meat sample were Tazaj beef luncheon, Esalat chelavra, Chicken luncheon altazaj althabi, Sardines, Silou tuna and Chicken

pieces nasma, where the number of colonies exceeded the permissible numbers, which exceeded the upper limisurrounding

Recommendations

1- Study the effect of S. aureus toxin in lab animals, and study the histopathological change and determined the lethal dose(LD) and infection dose (ID) of S. aureus toxin.

2-Investigate the effectiveness of some plant extracts in affecting the inhibition of bacterial growth when added to food.

3-Study the types of anaerobic bacteria in canned food and their resistance to antibiotics.

4-Study Staphylococcus lentus

Reference

Reference

Ababio, P. (2015). An investigation into the incidence of food pathogenic bacteria In senior secondary school canteens in the Ashanti region of Ghana and

The effect of food safety interventions (Doctoral diertation, University of Lincoln).

Abbott, S. L., Cheung, W. K. W., and Janda, J. M. (2003). The genus

Aeromonas: biochemical characteristics, atypical reactions, and

Phenotypic identification schemes. J. Clin. Microbiol. 41, 2348–2357. Doi:

10.1128/JCM.41.6.2348-2357.2003

Abdel-Tawab, A.A., El-Hofy, F.I., Maarouf, A.A. and Abbas, S.A., 2016.

Molecular detection of some virulence genes of S. aureus isolated from

Mastitic Cows by PCR. Benha Veterinary Medical Journal, 30(1), pp.238-245.

Adams, M. R., Moss, M. O., & Moss, M. O. (2000). Food microbiology. Royal Society of chemistry.

Aduah, M., Adzitey, F., Amoako, D. G., Abia, A. L. K., Ekli, R., Teye, G. A., … & Huda, N. (2021). Not All Street Food Is Bad: Low Prevalence of

Antibiotic-Resistant Salmonella enterica in Ready-to-Eat (RTE) Meats in

Ghana Is Associated with Good Vendors' Knowledge of Meat Safety.

Foods, 10(5), 1011.

Aggarwal S, Jena S, Panda S, Sharma S, Dhawan B, NathG, Singh NP, Nayak

KC and Singh DV. (2019).Antibiotic susceptibility, virulence pattern and

Typing of staphylococcus aureus strains isolated from variety of infections In India. Front Microbiol., 10: 2763.

Branen, A. L., Davidson, P. M., Salminen, S., & Thorngate, J. (Eds.). (2001).

Food additives. CRC Press.

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of Gram-Negative

Bacteria to Current Antibacterial Agents and Approaches to Resolve It.

Molecules (Basel, Switzerland), 25(6), 1340.

Buchanan, R. L., & Oni, R. (2012). Use of microbiological indicators for assessing hygiene controls for the manufacture of powdered infant formula. Journal of food protection, 75(5), 989-997.

Centers for Disease Control (US). (1980). Foodborne Disease Surveillance,

Annual Summary. Department of Health and Human Services, Public Health Service.

Centers for Disease Control and Prevention (CDCP), (February 22, 2022), Fast Facts About Food Poisoning.

Centre for Food Safety. (2014). Microbiological Guidelines for Food (For readyto-eat food in general and specific food items). In Food and Environmental Hygiene Department, Hong Kong.

Chapaval, L.; Moon, D. H.; Gomes, J. E.; Duarte, F. R. and Tsai, S. M. (2006). Use of PCR to detect classical enterotoxin genes (ent) and toxic Shock syndrome toxin-1 gene (tst) in Staphylococcus aureus isolated from Crude milk and determination of toxin productivities of S. aureus isolates Harboring these genes. Arq. Inst. Biol. 73(2):165–169.

Rugemalila, G. A. (2015). Investigations on Environmental Risk Factorof Responsible For High Prevalence of Food Borne Diseases: The case of Temeke Municipal, Dar es Salaam (Doctoral dissertation, The Open University of Tanzania).

Sahin S, Mogulkoc MN, Kalin R and Karahan M. (2020). Determination of the Important toxin genes of Staphylococcus aureus isolated from meat Samples, food handlers and food processing surfaces in Turkey. Israel Journal of Veterinary Medicine 75(2): 42–49.

Salasia, S. I. O., Khusnan, Z., Lammler, C., & Zschock, M. (2004). Comparative studies on pheno-and genotypic properties of Staphylococcus aureus isolated from bovine subclinical mastitis in central Java in Indonesia and Hesse in Germany. Journal of Veterinary Science, 5(2), 103-109.

Samaha, I.A., Ibrahim, H.A.A., Hamada, M.O (2012).Isolation of Some Enteropathogens from Retailed Poultry Meat in Alexandria Province. AJVS. 2012; 37(1): 17-22

Sambrook J, &Russell DW.(2006). Agarose gel electrophoresis. Cold Spring Harbor Protocols. Jun 1;2006(1):pdb-rot4020.

Şanlıbaba, P. (2022). Prevalence, antibiotic resistance, and enterotoxin production Of Staphylococcus aureus isolated from retail raw beef, sheep, and lamb Meat in Turkey. International Journal of Food Microbiology, 361, 109461.

Savariraj, W. R., Ravindran, N. B., Kannan, P., & Rao, V. A. (2021). Occurrence and enterotoxin gene profiles of Staphylococcus aureusYoon, S., Park, Y. K., Jung, T. S., & Park, S. B. (2022). Molecular Typing, Antibiotic Resistance and Enterotoxin Gene Profiles of Staphylococcus Aureus Isolated from Humans in South Korea. Microorganisms, 10(3), 642.

Yousif, L. M., Mohamed, A. K., Nor El-Deen, A. M., & Mohamed, R. E. S. (2022). Phenotypic Methods of Detection of Antibiotic Resistance in Gram Positive Bacteria in Sohag University Hospital. The Egyptian Journal of Hospital Medicine, 87(1), 1511-1516.

Zhang, P., Liu, X., Zhang, M., Kou, M., Chang, G., Wan, Y., ... & Wang, X.

(2022). Prevalence, Antimicrobial Resistance, and Molecular Characteristics of Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus from Retail Ice Cream in Shaanxi Province, China.

Foodborne Pathogens and Disease, 19(3), 217-225.

Zhou, J. (2021). Targeting Enzymes Involved in Antimicrobial Resistance (AMR) In Gram-negative Bacteria (Doctoral dissertation, UCL (University College London)).

Zouharova, M. and Rysanek, D., 2008. Multiplex PCR and RPLA identification Of Staphylococcus aureus enterotoxigenic strains from bulk tank milk.

Zoonoses and Public health, 55(6), pp.313-319.

Zulfakar, S. S. ; Abu-Bakar, N. F.; Aidilputra, A. A. ; Miatong, A. and Chong, E. S. (2019). Microbial Contamination of Meat Contact Surfaces At the Selected Beef Processing Plants in Selangor and its Biofilm Formation Ability. Pertanika J. Trop. Agric. Sc. 42 (2): 709 – 726.

[2]Bachoon، Dave S. ؛Dustman، Wendy A. (2008). "Exercise 8: Selective and Differential Media for Isolation". Michael Stranz Microbiology Lab

الخلاصة

سلامة الأغذية هي التركيز الأساسي لبيولوجيا الغذاء ؛ تعد البكتيريا والفيروسات والسموم المسببة للأمراض التي تنتجها الكائنات الحية الدقيقة من الملوثات الغذائية تم جمع تسع عينات من الأطعمة المعلبة (المربى واللبن والجبن) وتم جمعها المحتملة عشوائياً من محلات السوبر ماركت المختلفة في الحلة بالعراق. عند عزل العينات وزراعتها على الاجار المغذي كانت النتيجة بعد احتضانها ظهور مستعمرات بكتيرية ، وفي بعض العينات ، بعد زراعة العينات واحتضانها ، وجدت أن معظم العينات من الأغذية المعلبة تجاوزت العدد المسموح به في المعايير البيولوجية لسلامة الغذاء ، وتم أجراء صبغه غرام على العينات ، وتم مشاهده العينات الملطخة بصيغة الجرام تحت المجهر كانت موجبة لصبغه گرام كروية ستافيلوكوكس وعند تنميه البكتريا على وسط الايوسين منتول بلو كان مثبط ، لأن البكتيريا موجبة الجرام لا يمكنها النمو والتكاثر وتشكيل مستعمرات بكتيرية على وسط أزرق يوزين ميثيلين

لأنه خاص بالجرام السالبة وعند تنميه بكتريا على وسط المانتول سلت اگار وبعد

فترة الحضانة نلاحظ بكتيريا المكورات العنقودية (موجبة الجرام) تخمر المانيتول: لأن الوسط يأخذ اللون الأصفر.

وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية العلوم

قسم علوم الحياة

عزل وتشخيص البكتيريا المرضية من عينات الغذاء

بحث

مقدم الى مجلس كلية العلوم / جامعة بابل عام

كجزء من متطلبات نيل درجة البكالوريوس في الأحياء / الأحياء الدقيقة

بواسطة

آيات رسول عبد الحسين

إشراف

أ.د. ايمان محمد جارالله

١٤٤٤هـ ٢٠٢٣