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**College of Science**
**Department of Biology**

# Characterization of *Staphylococcus aureus* Isolated from meat samples in Hilla city

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of Sciences in Biology

By

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بسم الله الرحمن الرحيم

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ
أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ
خَبِيرٌ)

**صدق الله العلي العظيم**

«سورة المجادلة: الآية ١١»

# Certification

I certify that this thesis entitled(**Characterization of *Staphylococcus aureus* Isolated from meat samples in Hilla city )** was prepared By **Esraa Mohammed Kadhim Abdel Al Ameer** under my supervision at the Department of Biology, College of Science / University of Babylon, as a partial fulfillment of the requirements of Master of Science Biology –Microbiology.

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We certify that we have read this thesis entitled " **Molecular study Enterotoxin profile among *S. aureus* isolated from meat and meat product** " and as examining committee, examined the student " **Mareen Ismael Hassan Jassim** " in its content. In our opinion, it meets the standards of a thesis for the degree of Master of Science in Biology and accepted with "**Excellent**" degree.

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# Dedication

To my

dear father

soul of my mother

lovely husband

children *Rawan, Amir* and *Razan*

dear brothers and sisters

best friends who share happy and sad moments with me

I dedicate this work

Esraa 2022

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# Summary

Food safety is a vital component of ensuring a high standard of living. Food is one of the most basic need for human survival. It provides individuals with the energy and nutrition they needs to be healthy and productive. However, food that has not been thoroughly inspected may contain harmful bacteria. Contamination by microorganisms or chemicals might cause human health problems. These Foodborne infections, sometimes known as food poisoning, are a type of ailment that occurs when food is consumed. Food safety serves a number of purposes, such as to protect humans from foodborne infections.

All sample were plated on nutrient agar for total bacterial count . the isolates then cultured on MacConkey ,mannitol salt agar, blood agar, eosin methylene blue, and then submitted for Vitek 2 compact system For confirmation and then investigated antibiotic susceptibility was done according CLSI 2021. The Staphylococcucal Enterotoxin genes were investigated by polymerase chain reaction.

A total of 87 random samples, sixty samples each of Luncheon 15(17.24%), Tune 9(10.34%), Chicken pieces 9(10.34%), Minced meat 7(8.04%), Grilled meat 5 (5.74%), Hamburger 5(5.74%), Sausage 10(11.49%), and Fresh meat 27( 31.03%)from some supermarkets in Hila city in Iraq, were( 80) sample growth on nutrient agar and 7(8.04%) sample not growth from Fresh meat .

The obtained results showed that most bacteria diagnosed in these sample were *Staphylococcus aureus* 22(27.5%), *Enterobacter cloacae* 18(22.5%), *Burkholderia cepacia*1(1.25%), *Staphylococcus* lentu18(22.5%)s, *Klebsiella pneumonia* 12(15%), *Streptcoccus agalactiae* 3(3.57%), *Kocuria kristinae* 1(1.25%), *Micrococcus luteus* 4(5%) , *Gemella sanguinis*1 (1.25%).

The obtained results showed that Antimicrobial susceptibility testing of 22 isolates from *S. aureus* against different antibiotics clearly showed high susceptibility to Ciprofloxacin 22(100%), Levofloxacin 22(100%), Nitrofurantoin 22(100%), Gentamicin (90.9%) followed by Rifampicin(86.36%), while high resistance was observed against Penicillin 22(100%), Trimethoprim 22(100%), followed by Erythromycin (86.6% ), Clindamycin(86.6 %), Chloramphenicol (77.27%), Tetracycline(68.81 %), and Oxacillin (63.63%).

.Moreover, ES gene were detected in *S. aureus* isolates by molecular screening and appeared Total n=(22) of *S. aureus* the *sea* gene 7(31%), *seb* 9(40.9%), *sec* 5(22.7%), *sed* 2(9.09%), and *see* 2(9.09%). the presence of enterotoxin in *S. aureus* strains, particularly among processed meat products, portends the need for strict application of appropriate sanitary measures and Good Manufacturing Practices (GMPs) during food preparation, handling and storage.

The current study concluded that most frequent pathogen among meat and canned meat, was *Staphylococcus aureus* with different profile enterotoxin genes and its resistance to antibiotics.

## List of contents

### Chapter One: Introduction

### Chapter Two: Literatures Review

Chapter Four: Results and Discussion

## List of Tables

## List of Figures

## List of Abbreviations

| **Symbol** | **Description** |
|---|---|
| AST | Antibiotic sensitivity test |
| $a_w$ | Water activity |
| BHIA | Brain Heart Infusion Broth |
| CDCP | Centers for Disease Control and Prevention |
| CFU/ml | Colony forming unit per milliliter |
| CLSI | Clinical and Laboratory Standards Institute |
| D.W. | Distilled Water |
| DHHS | Department of Health and Human Services of the United States of America |
| DNA | Deoxyribonucleic Acids |
| E. coli | *Escherichia coli* |
| EMB | Eosin Methlene Blue Agar |
| FBDs | Food-borne diseases |
| $H_2SO_4$ | Sulfuric Acid |
| MDR | Multiple Drugs Resistant |
| MHA | Muller Hinton Agar |
| MRSA | Methicillin Resistance *Staphylococcus aureus* |
| MSA | Mannitol salt agar |
| PCR | Polymerase Chain Reaction |
| RTE | Ready to eat food |
| *S. aureus* | *Staphylococcus aureus* |
| SEA | Staphylococcal Enterotoxins A |
| SEB | Staphylococcal Enterotoxins B |
| SEC | Staphylococcal Enterotoxins C |
| SED | Staphylococcal Enterotoxins D |
| SEE | Staphylococcal Enterotoxins E |
| SEs | Enterotoxins |
| SFP | Staphylococcal Food Poisoning |
| TBE | Tris - Borate - EDTA |
| WHO | World Health Organization |

| ***Unit of Measurement*** | |
|---|---|
| **Abbreviate** | **Key** |
| **µg** | Microgram |
| **µl** | Microliter |
| **mV** | Millivolts |
| **g** | gram |

# *Chapter*
# *One*
# *Introduction*

## 1.1.Introduction

Foodborne diseases ( FBDs ) are the presence of physical, chemical, and biological factors in food that lead to disease in humans. Biological factors include: viruses, fungi, bacteria, and parasites. Bacteria are among the most important pathogens in humans. Diseases may be mild or fatal (Rortana *et al.*, 2021;Husain& Aziz, 2022)

Bacteria are classified according to their need for oxygen into aerobic , and anaerobic, and aerobic bacteria are classified into spore-forming bacteria, which are Bacillus, and they are very dangerous because their spores remain for a long time and resist high temperatures and bacteria that do not form spores. These bacteria are contaminated with food and infect humans with diseases ( Wang& Yuan ,2022).

To prevent possible food poisoning an examining food before is done distributing it to the consumer reduces the risk of poisoning and death. Food can be stored for long periods of time if proper procedures are followed, such as cleaning food contact surfaces, and preventing microbial enzymes from spoiling the food (Husain & Aziz, 2022).

Meat is of high nutritional value as it provides a protein for the consumer, which has a major role in building the human body as it provides amino acids. but during the slaughter and canning operations, meat is contaminated with various pollutants harmful to humans, it is a suitable environment for the growth of microbes ( Aduah *et al.*, 2021).

Meat includes two types of white and red meat, and the quality of these meat depends on several factors, including: temperature, humidity, pH, the cooking process and preservatives added to those meat and the presence of inhibitory and activating substances for microbes ( Hameed *et al.,* 2021).

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 (Pires *et ,al*, 2021 ;Mi, *et al*. ,2021; Aguirre Garcia *et al*.,2022; Husain& Aziz, 2022;) .

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 (Beier, 2021;Qi, 2022).

Due to little studies Staphylococcus Enterotoxin among meat and meat product and importance of food poisoning for human health so the current study aims to investigate the enterotoxin profile and antibiogram of *Staphylococcus aureus* isolated from meat and meat product.

The present study aim to isolate and identify different bacteria from meat samples and meat products collected from the different supermarkets and butcher shops in Babylon province and reveal the enterotoxin gene among *S. aureus* isolated. To reach this aims, via the following objectives: -

**1-**Collecting meat samples from local butcher shops and supermarkets.
2-Phenotypic and genotypic identification of bacteria from meat samples.
3-Molecular identification of *S. aureus* .
4-Studying the antibiotic susceptibility test of *S. aureus.*

5- molecular identification of enterotoxin(*sea , seb, sec, sed, and see*) profile of *S. aureus* .

# Chapter Two

# Literature Review

# 2. Literature Review

## 2.1.Food-borne diseases

Foodborne diseases are infections of the gastrointestinal system caused by ingesting foods or drinks that contain hazardous bacteria, parasites, viruses, or chemicals, according to the Department of Health and Human Services of the United States of America (DHHS) (Cliver& Riemann 2002; WHO,2015; Solis, 2016; Newby, 2018).

When two or more persons acquire the same symptoms after eating the same food, this is referred to as a foodborne illness outbreak (Centers for Disease Control and Prevention(CDCP), 2020; Nguyen, 2016).

Foodborne infections are more common in food service settings. Restaurants, schools, hospitals, college cafeterias, catering operations, bars, and businesses that serve meals to the general public are considered food services (Rugemalila,2015).

Because of the ideal environment in order to establish a broad range spectrum of food-borne illness microorganisms, the burden of food-borne diseases is predicted to be higher in developing nations WHO 2013. For instance, diarrheal illnesses claimed the lives of 2.1 million individuals in the year 2000 alone. According to the World Health Organization (WHO), children account for 90% of yearly diarrheal deaths, especially in underdeveloped countries (Rugemalila,2015).

The presence of physical, chemical, and biological elements in food that cause sickness in humans are known as foodborne diseases (FBDs). Biological components include , viruses, fungus, bacteria, parasites, and poisons, all of which make them inappropriate for human consumption. Bacteria are one of the most common infections in humans, causing a high rate of illness and mortality. Diseases can range from minor to severe Rortana *et al* .,2021 ;(Husain& Aziz, 2022) .

Examining food before giving it to the customer minimizes the risk of poisoning and mortality from food poisoning. If suitable processes are followed, such as cleaning food contact surfaces and preventing microbial enzymes from degrading the food, food can be preserved for lengthy periods of time (Husain , & Aziz, 2022).

Meat has a high nutritional value since it supplies the customer with protein, which has a crucial role in the human body's development because it contains amino acids. However, meat is polluted with many chemicals that are detrimental to humans throughout the slaughter and canning processes, creating an ideal habitat for the development of bacteria ( Aduah *et al.*, 2021; Arero & Abebe, 2022).

Microbial meat spoilage causes significant losses during the production, shipping, and storage of meat products, accounting for approximately 21% of all food losses. A thorough study of the characteristics of microorganisms linked with meat rotting is essential to minimize spoiling processes and develop innovative preservation treatments and methods for meat and meat products (Shao *et al.*,2021).

*S. aureus* is one of the most common causes of FBDs worldwide. *S. aureus* has been linked to a number of outbreaks, and food poisoning caused by *S. aureus* has been recorded in a number of countries and with a variety of foods. FBDs have been a source of worry for thousands of years, and ancient restrictions on clean and unclean food may have been based on scientific understanding of food safety. Consumer research in both developed and developing nations reveals a significant degree of worry about food safety (Gould *et al*., 2013)

*S. aureus* is linked to food poisoning as a result of ingesting enterotoxin-contaminated food (Johler *et al.,* 2016).

## 2.2.Meat

Beef, mouton, lamb, chicken, fish, and other types of meat are available, despite the fact that their compositions are similar, each variety of meat has a distinct worth (Lawrie & Ledward, 2014; De Smet and Vossen, 2016) .The term meat refers to meat, skeletal muscle, and all connective tissues except for bone marrow and bone. Meat is a high-protein food, vitamins and minerals. Meat is one of the most prevalent, healthful, and energy-dense natural foods consumed by people on a regular basis. Maintaining a healthy and balanced diet is seen as critical (Klurfelde, 2018). Meat is considered perishable because it gives a proper environment for the growth of microbes. Skeletal muscle is germ-free, but it gets contaminated during slaughter and transportation. therefore, meat-borne pathogens are considered to be the cause of disease and death ( Bantawa *et al.,*2018) .

There are different factors of physical, chemical, and biological agents can spoil meat, which are distributed as follows :

(1) microorganisms like bacteria .

(2) Lipases and proteases, among other enzymes, break down meat into smaller chains of amino acids. Peptides link amino acids together, whereas proteases break them apart.

(3) Browning and oxidation are two chemical processes that occur in meals.

(4) freezing, melting, drying, crushing, and exerting pressure are examples of physical changes. (Iulietto *et al*., 2015; Erkmen and Bozoglu, 2016)

Bacteria are the most prevalent cause of animal food quality degradation, own to the fact that a range of factors have been linked to meat rot. Fats, carbohydrates, and proteins in meat are broken down by rotten bacteria, leading to a loss of flavor, slime production, and discoloration, rendering the meat unsuitable for human consumption (Pellissery ,2020).

## 2.3. Foodborne Pathogens associated with meat

Food poisoning is another term for foodborne bacteria. It occurs as a result of eating germs found in food, and these germs are viruses, fungi, parasites, and bacteria. Bacteria cause food spoilage or food poisoning. Microorganisms require nourishment for a variety of reasons, including survival and the ability to migrate to other organisms, such as people and animals. Bacteria that grow on food are divided into three categories: helpful, spoilage, and disease-causing microorganisms (Adams *et al*.,2000).

*Lactobacillus acidophilus*, *Lactobacillus plantarum,* and *Streptococcus thermophiles* are examples of beneficial microbes found in the fermentation of foods like cheese and pickles. Microorganisms that alter the content, flavor, or color of food are frequently not harmful, but they do cause financial losses. *Alicyclobacillus, Brochothrix, Bacillus,* and *Clostridium* are examples of food spoilage microbes. Microorganisms that cause food poisoning , they mostly grow on food and do not change anything content or color of the food, but they do cause illnesses in people and animals for example *S. aureus* (Bhunia, 2018).

There are more twenty-five genera of bacteria that cause food spoilage and diseases, as shown in Table (2-1) (Umar, 2021).

**Table 2.1 Bacterial genera associated with foodborne infections or food intoxication(Umar, 2021)**

| Gram-positive | Gram-negative |
|---|---|
| *Bacillus, Clostridium, Listeria Brucella , Mycobacterium, Staphylococcus , Bacilli, Pediococuus*<br>*Corynebacterium, Lactobacillus*<br>*Micrococcus, Pedicoccus ,Streptomyces, Leuconostoc* | *Aeromonas , Arcobacter , Campylobacter*<br>*Enterobacter , Cronobacter, Escherichia , Salmonella, Shigella, Vibrio, Yersinia , Serratia , Alcaligens, Bacteroids, Citrobacter , Erwinia ,*<br>*Flavobacterium , Acetobacter* |

## 2.4.Factors Determining the Growth of Bacteria in Food

Foods can be classified as : (Erkmen and Bozoglu, 2016; Pellissery,2020).

(1) Beef, poultry, milk, and eggs are very perishable, with a high level of retention duration of 13 days.

(2) “Semiperishable foods” include eggs, pasteurized milk, and foods that need to be kept cool with a 13-week shelf life.

(3) Vacuum-packed beef and sausages that have been fermented with a 14-month shelf life are examples of semi-shelf stable items.

(4) Processed foods that have been shelf-stable, such as vacuum-packed beef and fermented meals these meals can be consumed and stored for up to a year in good condition. Just a few examples are dried foods, wheat, and sugar.

Beef and meat products are characterized as perishable as a result of the following features: (Dav and Ghaly, 2011; Erkmen and Bozoglu, 2016; Magar, 2021)

1. A high level of water activity at least 0.85.

2. A high nutritional content that encourages the growth of microorganisms

3. a pH of at least 4.6

4. Autolytic enzymes are present

5. A considerable quantity of fat and lipids present, which favors oxidation and spoiling.

## 2.4.1-Intrinsic factors

The internal factors are related to the food itself and include several factors (Bhat *et al.,* 2012;Preetha & Narayanan 2020).

### 2.4.1.1. pH

A food's pH value is a logarithmic measurement of its acidity. Since ancient times, increasing the acidity of foods has been utilized as a preservation strategy, either by addition of mild acids or fermentation. Most meals, for example meat, vegetables, and fish, are somewhat acidic in their natural form, whereas the majority of fruits have a mild acidity some foods, including egg white, are alkaline. ( Hernández-Cortez *et al.,* 2017).

The relationship pH = -log [H +] defines pH Because each pH unit reflects a 10-fold difference, a meal with a pH of 6 is 10 times more acidic than a food with a pH of 7, and a food with a pH of 5 is 100 times more acidic than a food with a pH of 7. (Matthews *et al.,*2017).

Bacteria have a narrower pH range of growth, Microorganisms are categorized according to their pH ranges ( Bhat *et al.*,2012; Umar, 2021)

Microbes are classified as Acidophiles, Neutrophiles, or Alkaliphiles depending on their ideal pH for growth.

1-“ Neutrophiles thrive at a pH range of 5–8.”

2- “Acidophiles thrive in environments with a pH below 5.5.”

3- “At a pH of above 8.5, alkaliphiles thrive.”

Bacteria differ with respect to their ideal pH. (Kim *et.al*.,2019)

### 2.4.1.2. Water activity ($a_w$)( Moisture content)

It is the Quantity of water available to perform the biological functions of the bacteria cell for the purpose of growing on food. Bacteria need water for the purpose of growing on food, taking it in several forms, including solutes and ions, Hydrophilic colloids, the water of crystallization , the water activity of meal may be calculated by comparing the meal's water at the same temperature, with the vapor pressure of pure water (Tapia *et al.*,2020).

“$a_w = p/p_o$”

where p = the solution's vapor pressure

$p_o$ = vapor pressure of the solvent (usually water)

Its range is from 0 to 1( Bhat *et al*., 2012) . Only *S. aureus* can thrive and manufacture toxins $a_w$ 0.90 or less, which is why many bacterial diseases are managed at water activity much over 0.86. The $a_w$ values of most fresh items, such as fresh meat, vegetables, and fruits, are close to the optimal development level of most bacteria (0.97 - 0.99) .Various foods and their water activities are: Fresh poultry or fish(0.98-1.00), Fresh meat(0.97), Eggs(0.91-1.00) (Magar, 2021)

Bacteria do not grow below 0.91and bacteria that are gram-negative are more vulnerable to a minimum water activity than gram-positive bacteria.

According to the $a_w$, bacteria were divided into the following groups: (Bourne, 2017).

1-“Aerophils :They are bacteria that grow in dry conditions”.

2-“Halotophils : These are bacteria that grow in high salt concentrations”.

3-“Osmophils : which can be grown in the presence of high organic compound concentrations such as polysaccharides”.

Water binding to various macromolecular components in the meal, adding solutes like salt or sugar, removing water physically by drying or baking, or adding water to various macromolecular components in the meal are all ways to modify the $a_w$ in foods (Syamaladevi *et al.,*2016; Wolf-Hall & Nganje, 2017).

## 2.4.1.3. Oxidation-reduction potential (Eh)

Meat is a food rich in nutrients represented in fats and proteins and is susceptible to oxidation and therefore meat damage occurs as a result of oxidation and reduction (Faustman, 2010).

A substance's oxidation-reduction potential (or redox potential): It is the transfer of electrons between atoms or molecules and measured in terms of millivolts (mV). Food oxidation-reduction is influenced by its pH , capacity for posing (or buffering capacity), composition of the diet (such as ascorbic acid, a protein, , and reducing sugars), and pH ( Bhat *et al.,* 2012).

Bacteria can be divided according to Eh: Aerobes , Facultative anaerobes, Anaerobes

## 2.4.1.4. Nutrient content

Food may be used by microorganisms as a source of nutrients and energy. They get the chemical components that make up microbial biomass, the molecules that the organism cannot generate for growth, and the substrate that can be utilized as a source of energy from it. Meat or casein (peptone and tryptone), meat marinating, tomato juice, malt extract, sugar, and starch are all commonly used in microbiological media, indicating their aptitude for this purpose ( Adams *et al.* ,2000 ) .

Bacteria need nutrients such as protein, carbohydrates, vitamins, salts, minerals, etc. to grow and perform metabolic functions. Gram-positive bacteria have higher nutritional requirements than Gram-negative bacteria (Rolfe and Daryaei, 2020). Being unable to use a key component of a dietary material will limit an organism's development and put it at a disadvantage in comparison with those who can. As a result, an organism's capacity to generate amylolytic (starch-degrading) enzymes will help it thrive on grains and other farinaceous items. When fruits containing sucrose and other sugars are added to yoghurt, the spectrum of carbohydrates available expands, allowing yeasts to create a more diversified spoilage microflora ( Adams *et al.* ,2000 ).

## 2.4.1.5. Presence of antimicrobial constituents

There are some foods that contain substances within their composition that are resistant to bacteria (Jay *et al.*,2008). Essential oils are substances that have been extracted from plants (eugenol in cloves and cinnamon , cinnamic aldehyde in cinnamon ,Thymol in sage, eugenol in cloves and cinnamon, allicin in garlic)are found in spices, lactoperoxidase, lactaferrin, , and ovoflavoprotein in hen's egg albumin ,and lysozyme in milk, and, avidin, lysozyme,) can help foods resist microorganism. Similarly, casein and free fatty acids present in milk have antibacterial properties. Antibacterial and antifungal action is found in hydroxycinnamic acid derivatives (chlorogenic acids feluric, and p-coumaric caffeic) found in vegetables , tea ,fruits, and other plants. Natural coverings of foods, such as the shells of eggs and nuts, the outer coverings of fruits and seeds, and animal hides, also protect against invasion and subsequent spoiling.

## 2.4.2. Extrinsic factors

These are the external factors that control the growth of bacteria. (Rolfe and Daryaei ,2020).

### 2.4.2.1.Temperature of storage

Temperature greatly influences bacterial growth and enzymatic reactions (Tornberg, 2005). Depending on the environment temperature, bacteria are divided into several categories (Lawrie, 2014; Iulietto *et al*., 2015; Umar, 2021)

"Psychrotrophs": It is a cold-tolerant bacteria within the range (0°C-20°C) includes *Enterococcus spp*. and *Pseudomonas spp*

"Mesophiles" : It is a bacteria that grows at a temperature of (25°C-40°C) and the optimum temperature for it is 37°C and it includes species of *Staphylococcus, Salmonella, Shigella, Bacillus,* and *Clostridium.*

"Thermophiles": It is the bacteria that temperatures over 45°C are ideal for growth, and the optimum temperature for it is between 50°C and 70 °C. Among them are the species of *Clostridium, Bacillus,* and *Geobacillus.*

### 2.4.2.2.Relative Humidity

It is the quantity of water in the air (Atmosphere) that promotes bacterial growth, such as dry grains, it is more resistant to bacteria, but when absorbing moisture in the air, it exposes it to the growth of bacteria on it (Yang *et al*., 2009)

### 2.4.2.3. Gases in the environment: their presence and concentration

These are gases that inhibit or kill bacteria such as Carbon dioxide ($CO_2$), ozone ($O_3$), and oxygen ($O_2$). Ozone and oxygen are poisonous gases for anaerobic bacteria and $CO_2$Acts against aerobic compulsive bacteria.( Lee, 2021).

## 2.5.Bacteria in raw meat

In healthy live animals, muscle tissue is virtually sterile. Raw meat contains a high content of nutrients and moisture. When exposed to ambient conditions, raw meat is liable to spoilage without preservation treatment, reflected by the changes in color, odor, etc. (Ren, *et al.*, 2021). Thus, the physiological state of the animal at the moment of butchery, the spread of pathogens during animal slaughter , a number of factors affect the early microbial load and composition of fresh meat , including the slaughterhouse environment (Erkmen and Bozoglu, 2016; Zulfakar *et al* ., 2019) . Once the carcass has been opened, and the offal is removed after slaughter, the major source of meat contamination occurs. Meat can be contaminated by germs derived from skin, stomach, lymph nodes, processors, cutting blades, and a processing facility, for example the micro biota of a slaughterhouse, which is a combination of bacteria throughout the facility and in the intestines of the animals, is a term for these microorganisms gained through meat(Bhunia, 2018). Mills and colleagues demonstrated that Carnobacterium spp. found in lamb carcasses could be traced back to a meat-processing environment (Mills *et al.*, 2018) . The primary bacteria in the slaughterhouse, according to microbial diffusion research include Psychrobacter spp., Acinetobacter spp, Streptococcus spp., Staphylococcus spp, Brocothrix spp., and LAB. Proteobacteria, members of the Enterobacteriaceae family and particularly Pseudomonas spp. were identified to be the most prevalent microbes in the carcass micro biota (Koutsoumanis and Sofos, 2004) .

On the carcass, the first micro flora is varied with a higher proportion of Mesophiles than Psychrotrophs. On fresh beef, the overall microbial burden varies from $10^2$ to $10^5$ CFU/cm2. Clostridium, Bacillus spp, Flavobacterium, Achromobacter, Cytophaga, Aeromonas, Moraxella, Acinetobacter, Brochothrix thermosphacta, Enterobacteriaceae including E. coli, Micrococcus, LAB, Staphylococcus, Pseudomonas, and Streptococcus make up the first microbiome(Pellissery,2020). The micro biota that finally forms on meat and causes spoiling is determined by the product's packing technique. Meat that has been freshly prepared is packaged using one of three methods: aerobic, vacuum, or modified environment packaging(MAP) (packing for a changed environment ). The presence or lack of oxygen in the microenvironment around beef packed impacts the final makeup of the meat microbiome and determines its shelf-stability(Farkas,2007). When the bacterial density exceeds $10^8$ cells/cm2 under aerobic circumstances, the first indicator of deterioration, notably off-odor, appears (Pellissery,2020).

## 2.6.Bacteria in cooked meat

The process of cooking food is one of the very important processes to kill bacteria that are contaminated with food or that are few in number, depending on the temperature used and the time sufficient to kill these bacteria. (Miraglia, 2009).

When cooked food is stored in a warm environment, even if the initial amount of bacteria is small, it can multiply rapidly within 8-12 hours of storage (Newman ,2005).

## 2.7. Bacterial food poisoning prevention and control measures

To prevent food poisoning by bacteria contaminating meat or to reduce the number of contaminated bacteria, there are several procedures that must be followed by people in canning factories, butcher shops, and even at home:

Basic food safety

1-The five keys for safer food developed by the WHO capture the basic principles of food safety, maintain a clean environment, keep raw and cooked foods separate, fully cook the food maintain a safe temperature for food and ,make use of clean water and materials .(Ababio, 2015).

2-The surface on which the meat is cut should not retain bacteria, that is, made of alloys containing antimicrobials, and should be easy to clean (Dudman, 2013).

3- Meat must be fully cooked before eating, and not left outside for more than four hours after cooking, as it must be stored in the refrigerator after it cools directly, or eaten four hours before its preparation (FONG, 2017)

4- One way to keep food fresh is to use that each microbe has an optimum temperature for its growth. When controlling temperatures above or below the optimum temperature, we will reduce microbial growth. For example, when lowering the temperature (refrigeration and freezing) it will disrupt microbial growth or raising the temperature (pasteurization and sterilization) will reduce the effectiveness of microbial growth. (Xiong, 2017)

5- There are some measures used as a minimum to inactivate microorganisms or enzymatic activity, namely:" (high hydrostatic pressures, ionizing radiation, ozone pulsed electric fields, pulsed light and UV light, ultrasound), or are under development (dense-phase carbon dioxide electric arc discharges and cold plasma, oscillating magnetic fields and pulsed X-rays)." (Fellows, 2017).

6- Food poisoning prevention in four steps

a)Wash your hands and work surfaces before, during, and after preparing meals. Germs may flourish on your hands, utensils, chopping boards, and surfaces, among other things. b) Separate raw meat, poultry, fish, and eggs from ready-to-eat meals. Raw meat should be stored apart from other foods in your shopping cart and refrigerator, and should be chopped on separate cutting boards. c) Cook food to the right internal temperature to kill harmful microorganisms. Check the temperature using a food thermometer. and d) Keep the temperature of your refrigerator below 40 degrees Fahrenheit. Within 2 hours of cooking, refrigerate leftovers (CDCP,2022).

7- The main factor for meat pollution was improper butcher shop requirements, stockpiling (raw meat stored in retail shops without \temperature control units), lousy transport, cross-contamination of meat and poultry, unsanitary conditions ideal for various microbial and microscopic pathogens, untidy freezing units ,and improper cooking (Ucar *et al.*, 2016).

## 2.8.Types of bacterial toxins

Toxins are potent factors that cause disease generated by bacteria, fungi, animals, and plants, and they mediate complex interactions between pathogens and their hosts. Bacterial toxins were the first substances to be discovered as the cause of serious bacterial infections in humans and animals. Bacterial toxins are extremely diverse in terms of structure (mono, binary, ternary, or multiple complex proteins), mode of secretion (different bacterial secretion types), size (ranging from 15 to over 2700 amino acids), membrane/intracellular receptor recognition, specific mode of action (pore-forming activity, for example), or enzymatic activity (Popoff,2018). Pathogenic bacteria cause damage to the host in two ways, either by invasive or by toxins (Henkel *et al*., 2010). Invasiveness is the ability of bacteria to grow in the host either inside or outside cells, where they secrete extracellular enzymes. As for bacterial toxins, they are caused by pathogenic bacteria that are often non-invasive or have a limited ability to infect the host, and toxins produced by bacteria are common which work away from the infection's source and regulate toxins physically as they recognize the receptors on sensitive cells and have an enzymatic ability to modify the target of the cell host. Bacterial toxins are classified according to the way they are released ,There are two types of bacterial toxins which are endotoxins and exotoxins. Endotoxins or lipooligosaccharide (LOS),or lipopolysaccharides (LPS): Gram-negative bacteria's outer membrane contains these components; it is thought to be the most significant antigen in bacteria.; It is released after the bacteria die or divide. This endotoxin is capable of causing endotoxin tissue damage and shock and heat labile ( Malangu,2017; Hernández-Cortez *et al.*, 2017)

The **exotoxins** that are released during the life of the bacterial cell. It generally consists of two parts of polypeptide chains, one component . The toxin's enzymatic activity is controlled by (subunit A) and the other component (subunit B) is in charge of attaching to a particular receptor on the host cell membrane and moving the enzyme across it. (one of which binds to the host B-subunit cell and the other is responsible for giving the A-subunit toxic effect, be unstable in temperature ( heat labile ). As shown in the figure(2-1) (Henkel *et al*., 2010).

A disulfide bond or non-covalent interactions may join the A and B domains together.

Some types of toxins produced by some foodborne bacteria: Enterotoxins (A, B, C1, C2, D and E, G, H, I, J), Toxin A/Toxin B (Clostridium difficile), Thermolabile toxin (LT), Thermostable toxin (ST) (Enterotoxigenic E. coli), Alpha-Toxin, Beta-Toxin, Epsilon-Toxin and Iota-Toxin (C. perfringens),Cholera toxin (Ctx) (***Vibrio cholerae***), Citotoxin K or CytK (Bacillus cereus), Shiga Toxin (Shigella dysenteriae and E. coli O157:H7), Cereulide ,CPE Enterotoxin (Clostridium perfringens), Botulinum toxin (BTX) (***Clostridium botulinum***), Toxic Shock Syndrome Toxin (TSST-1), and hemolysin BL (HBL), nonhemolytic enterotoxin (NHE) (***S. aureus)*** ( Malangu and Ntambwe , 2017).

## 2.9. Staphylococcal enterotoxins

*Staphylococcal* enterotoxins (SEs) are generally classified in a family of 20 different exotoxins that share a common phylogenetic relationship, structure, function, and sequence homology produced by staphylococcal and streptococcal exotoxins. Currently, 23 enterotoxins have been identified as distinct serological entities (Schlievert and Case, 2007) and these includes *Staphylococcal enterotoxin* A (*sea),* *Staphylococcal enterotoxin* B (*seb*), *Staphylococcal enterotoxin* C (*sec*), *Staphylococcal enterotoxin* D (*sed*), and *Staphylococcal enterotoxin* E (*see*).

These toxins are basic proteins comprising of about 220–240 amino acids and molecular weights of about 25–30 kDa. Staphylococcal enterotoxins *seb* and *seb* are the most common. *sea* is the most frequent cause of food poisoning caused by staphylococcus (Pinchuk *et al.*, 2010). *seb* has also been identified biological weapon of war and bioterrorism in addition to causing food poisoning and has therefore been restricted (Greenfield *et al*., 2002). Common among the foods that can be contaminated by SEs are dairy products, including milk.

Production of SEs rapidly increases at optimum temperatures (20–37$^o$C) and pH (4–7.4) (Greenfield *et al.*, 2002). Children suffer SFP by ingesting as little as 100 ng of SEs, and vulnerable populations may develop staphylococcal food poisoning with a few micrograms of toxin (Larkin *et al.,* 2009).

The mechanism of action and pathophysiology of SE-induced food poisoning has not been fully elucidated. However, it is hypothesized that the stimulation of the vagus nerve in the abdominal viscera by the SEs followed by transmission of the signals to the vomiting center in the brain, is responsible for vomiting in cases of SFP. Furthermore, the cholinergic receptors located on the afferent vagal neurons are key in *sea*-induced emesis (Hu et al., 2007), and that capsaicin from chili peppers has been found to deplete peptidergic sensory nerve fibers, leading to diminished SE effects in mammals (Larkin et al., 2009). Moreover, SEs can penetrate the gut epithelium and activate both local and systemic immune systems (Shupp et al., 2002). Consequently, mediators of inflammation released such as neuroenteric peptide substance P, histamine and leukotrienes, leads to vomiting. The resulting emesis can be diminished by H2- and calcium channel-blockers, through blockade of histamine release. In addition, the observed gastrointestinal damage may as well be caused by local immune system activation as a result of SE ingestion. Inflammatory lesions are commonly found in various regions of the GI tract with the jejunum and ileum showing the most severe lesions (Banwell and Sherr, 1973). The diarrhea associated with SEs induced SFP results from diminished reabsorption of water and electrolyte in the small intestine.

Some studies have shown that SEs do not directly act on the gastrointestinal tract, but indirectly affect the expression of cytokines and metabolites produced by T cells, macrophages, monocytes and mastocytes (Marrack and Kappler, 1990; Kotzin et al., 1992).

It has been postulated that superantigenicity and enterotoxicity is a result of enterotoxin activity facilitating transcytosis, leading to entrance of SE toxin into the bloodstream, hence facilitate the interaction with antigen presenting- and T-cells ultimately leading to superantigen activity (Balaban and Rasooly, 2000). Therefore, systemic invasion of SEs following ingestion and their spread from a localized *S. aureus* infection site, could have more severe effects upon the host than when the toxin remains localized (Larkin et al., 2009).

## 2. 10.Risk factors associated with food poisoning

The following are the top five dangers that you should be aware of are the most frequently responsible for incidents of foodborne illness, as determined by the CDC (Centers for Disease Control (US), 1980; Domino, 2013; Ncube *et.al*., 2020).

1. Holding temperatures of potentially dangerous food are too hot or too cold.
2. Food cooked at incorrect temperatures
3. Utensils and equipment that are dirty and/or polluted
4. Employee health and hygiene are poor.
5. Food obtained from untrustworthy sources

Because their immune systems are less effective, certain groups of people are more vulnerable to foodborne illness. The following are examples of such organizations ( McLauchlin *et al.*,2007; Hernández-Cortez *et al.*, 2017)

- Patients with cancer.
- Children under the age of 5.
- Patients with Diabetes.
- Patients with HIV/AIDS.
- Adults in their fifties and sixties.
- Autoimmune disease sufferer.
- Women who are expectant.

These at- risk groups should take extra precautions when consuming or preparing food due to their inability to fight infectious diseases like food poisoning (Garfunkel *et al.*,2007;Makun, 2016).

Among the most dangerous foods are fresh uncooked foods such as fruits and vegetables, as well as animal products such as raw meat and poultry, unpasteurized milk, dairy products, raw seafood, as well as raw eggs. The origin of microbes is very important in foodborne diseases, as there are very dangerous microbes such as botulism to medium severity such as staphylococcus ( Branen *et al*.,2001). When one or more of these danger variables are present, the likelihood of a foodborne illness epidemic is considerably increased whether food is prepared at home by the general public or by a food service provider , it is when one of the risks in retail food stores which is discovered, a serious violation that must be dealt with right away. Frequently, to reduce the risk of foodborne disease among the general public, the change necessitates the destruction of food goods (Guerrant, *et al*.,2011).

1) Temperatures of possibly hazardous foods that have been stored at dangerously high or low temperatures.(Collins,1997;Branen *et al*.,2001;King, 2013; Musa *et al*. 2010;Potter, 2021).

2)Foods cooked at incorrect temperatures ( Hernández-Cortez *et al.,* 2017; Malangu, 2016).

3) Utensils and equipment that are dirty or contaminated (McLauchlin *et al*.,2007;Hernández-Cortez *et al.,* 2017 ;Shumaker *et al.,* 2019;Mekonnen *et al.,*2021).

4) Employee Health and Hygiene Issues (Lubis *et al*,2019).

5) Unsafe Sources of Food(Stanfield, 2009).

## 2.11.Microbiological criteria for ready-to-eat food

Microbiological standards are guidelines for assessing the acceptability of foodstuffs and their manufacturing processes, and the most important of these procedures are hygiene and good manufacturing as they ensure food safety. In general, there are three components to the microbiological limits for ready-to-eat food (Centre for Food Safety,2014).

1-ACC stands for Aerobic Colony Count.

2- *E. coli* and Enterobacteriaceae are hygiene indicator organisms.

3-Ten distinct bacterial pathogens are found in foodborne pathogens.

The microbiological quality of ready-to-eat food will be classified into one of three classes1 based on the above three components.

(a) Satisfactory: test findings indicate that the microbiological quality is satisfactory.

(b) Borderline: test findings that are neither unsatisfactory nor satisfactory, are at the top limit of tolerance, and suggest the possibility of public health concerns and unacceptable risk.

(c) Unsatisfactory: For ACC, test findings indicating that the cause of the elevated count is being investigated and may be considered. Test findings for hygiene indicator organisms that need remediation. Pathogen test findings at levels that suggest a product that is potentially harmful to human health and/or unsafe for human consumption, necessitating prompt remediation.

**Table(2.2): Interpreting the findings of the ACC levels [30°C/48 hours] in various ready-to-eat food (Centre for Food Safety, 2014)**

| **Food Category** [a] | **Examples** | **Result (colony-forming unit (cfu)/g)** | | |
|---|---|---|---|---|
| | | **Satisfactory** | **Borderline** | **Unsatisfactory** |
| 1. Canned, bottled, cartoned, and pouched goods are ambient stable as soon as they are removed from the container. [b] | Salmon, Tuna, Sweets, Stews, Corned beef, Soups, and fruit are Canned, as are Ultra-high-temperature (UHT) items. | **$<10$** | **N/A** | **Note** [c] |
| 2. Foods that have been prepared just before being sold or consumed. | After regeneration, burgers, kebabs, takeaway food, sausages, ready meals, pizza (cook/freeze and cook/chill), dim sum, rice, and noodles | **$<10^3$** | **$10^3$-$<10^5$** | **$\geq 10^5$** |
| 3. Cooked foods that must be refrigerated after being sold or consumed; canned pasteurized foods that must be refrigerated after being sold or consumed. | Sausage rolls, Whole pies, flans, chicken portions quiches, samosas; pasteurised foods including Soups; canned ham requiring refrigeration; desserts and fruit juice | **$<10^4$** | **$10^4$-$<10^7$** | **$\geq 10^7$** |
| 4. Powdered meals, bakery and confectionery items without dairy cream | powdered dairy products, milk powder, Soup powders, and other reconstituted powdered meals ready to eat after reconstitution or warming | **$<10^4$** | **$10^4$-$<10^6$** | **$\geq 10^6$** |

| | | | | |
|---|---|---|---|---|
| 5. Cooked meals should be kept in refrigerated although they may need to be handled before being sold or consumed. | sliced pies, salad-free sandwiches, ho smoked fish (mackerel, etc. ), Sliced meats, pâté, mollusks, crabs, and other shellfish in shell, non-prepackaged cold drinks with solid ingredients but no dairy components (iced green tea with red bean, etc.) | **$<10^5$** | **$10^5$-$<10^7$** | **$\geq 10^7$** |
| 6. Mayonnaise and mayonnaise-based salads, prepared sauces, non-fermented dairy products ,and dairy desserts. | fresh cheese (paneer ,mascarpone), Most butter, dairy cream cakes,satay, dairy cream trifle, and non-prepackaged cold drinks with dairy components (iced milk tea with pearl tapioca, etc.)and solid ingredients | **$<10^5$** | **$10^5$-$<10^7$** | **$\geq 10^7$** |
| 7. Food that has been blended with dressings, dips, and pastes. | Houmous, dips, taramasalata ,cole slaw | **$<10^6$** | **$10^6$-$<10^7$** | **$\geq 10^7$** |
| 8. Refrigerated food products with a long shelf life. | Vacuum-packed or modified atmosphere packaging (MAP) items, such as fish, meat, vegetables ,and fruits. | **$<10^6$** | **106-$<10^8$** | **$\geq 10^{8\ d}$** |
| 9. Meat and fish that is raw and ready to eat, as well as cold smoked fish. | smoked salmon,Sushi, gravalax, sashimi, | **$<10^6$** | **$10^6$-$<10^7$** | **$\geq 10^7$** |
| 10. Pickled, marinated, or salted foods are examples of preserved foods. | salted fish, or Pickled, vinegar-cooked shellfish, spices, vinegar-cooked vegetables, and herbs, | **N/A** | **N/A** | **N/A** |

| | | | | |
|---|---|---|---|---|
| 11. foods that have been dried | spices, Herbs, dried fish, vine fruits, berries, nuts, herbs, spices sunflower seeds. | N/A | N/A | N/A |
| 12. Produce includes raw vegetables, fresh fruits and vegetables | Salads, mixed commodities salads comprising raw vegetables, sandwiches with salad, non-prepackaged cold drinks with solid and fresh fruit elements (sago and chilled fresh mango juice with pomelo, etc.) | N/A | N/A | N/A |
| 13. Meats that have been cured, fermented, and dried, as well as ripened cheeses and fermented vegetables | jerky and Continental sausages, salamis, sauerkraut, olives, cheddar, bean curd, brie, butter, stilton, and fermented milk beverages, yoghurt, and other dairy products | N/A | N/A | N/A |
| 14. Cooked meat items that may be displayed for a brief time at room temperature for sale, such as siu mei and lo-mei | Roasted ducks, roasted pork from China, and Poached chickens | $<10^5$ | $10^5$-$<10^6$ | $\geq 10^6$ |

N/A stands for "Not applicable." Notes: a. The ACC level of food products that are not included in these food categories should be evaluated by considering the raw components utilized, as well as the nature and degree of processing prior to sale. a. While most items are sterile when taken straight from the container, if they are ingested after further preparation, they are classified as Category 5. c. If spore-forming anaerobes are present, these items are rated as 'Unsatisfactory,' but they must be detected and counted using specific procedures. Spore-forming aerobes are normally absent in meals cooked in their packaging, although low quantities may be present in canned fish items. d. Keep an eye out for indicators of spoilage. Lactic acid bacteria thrive at low temperatures and do not thrive in an aerobic environment. Due to the formation of lactic acid, spotting will ultimately appear at a level of roughly 109 cfu/g. At 107 to 108 cfu/g, taints, discoloration, and slime created by pseudomonads, as well as slime produced by other Gram-negative bacteria, spoiling is likely to be visible, such as taints, discoloration, and slime produced by other Gram-negative bacteria.

## 2.12.Hygiene indicator organisms:

Bacteria that are utilized as a marker of quality or hygienic status in food, water, or the environment are known as indicator organisms. (Chapin *et al* .,2014; Martin *et al*., 2016)..In fact, the concept of an indicator organism is included in the definition of the word "indicator" which is defined as "anything so closely related with particular conditions that its presence is suggestive of the existence of these conditions." Historically, these issues have been linked to issues with sanitation and public health. However, throughout time, the use of indicator organisms has expanded to include evaluations of the quality, as well as the safety, of certain commodities ( Ashbolt *et al*., 2001).

The indicator tests have several desired properties that are considered (Buchanan and oni, 2012; Robinson,2014).

1- It is usually non-pathogenic, however pathogenic organisms are occasionally utilized (e.g., *S. enterica* in meat and poultry).
2- The detecting methods should be quick, widely available, low-cost, and simple to understand.
3- Indicator microorganisms appear at an appropriate frequency and level when the food safety system is under control.
4- Whenever possible, decision criteria should not be set at a minimum to detect the methods used.
5- Decision criteria for a microbiological indicator must be relevant at multiple points along the food chain.
6- The growth, inhibition, survival or resistance of a microorganism in a food must be at least equal to or greater than a potentially pathogenic microorganism that has an increased risk of occurrence under conditions that result in elevated levels of the indicator (eg), the growth of organisms The minute is an indicator in foods that

are abused at temperatures and the indicator remains in a dry product)

7- The choice of indicator tests should be based on a proper understanding of the characteristics and sources of microbiological hazards that the food safety system uses for control

They are of three types:

- “General microbial indicators (practical)”
- “Stool indicators (eg Escherichia coli)”
- “Index of objects and model objects.”(Ashbolt *et al*.,2001)

Food technicians divide the microorganisms in the indicator into two types, one section on food quality and another section on food safety.

1. Indicator Microorganisms for Food Quality (Halkman, 2014).

2. Indicator Microorganisms for Food Safety (Halkman, 2014).

## 2.13.Most commonly used indicator organisms in foods

Most types of microorganisms in food are the sum of viable cells, the sum of fungi and yeasts, Enterobacteriaceae , colon bacteria, and fecal bacteria. The presence of one of these indicators indicates poor sanitation and food handling or contamination after production.

When coliforms are present in normal levels in food, they can be killed by treatments such as radiation, pasteurization and ozone treatment. but when it is present in the final product, this indicates contamination (Robinson, 2014).It shows the relationships between the different groups of organisms discussed in this topic.

## 12.13.1.Total viable cell count

The number of microbial groups varies according to the sample and the treatment conditions, as the untreated samples contain large numbers of microbes, while the heat-treated types contain heat-resistant bacteria or resistant vegetative cells ( Robinson,2014). The total number of countable cells provides an estimate of the number of cells that can be recovered under experimental conditions (Heredia, 2009). This test shows a measure of the quality of the food, but the measurement of the total number of cells is weak because it is not directly related to pathogenic microbes or their toxins. The presence of low numbers of cells does not mean that the food is free of pathogens, and some foods contain high numbers of cells. This may indicate possible diseases and that there are problems in sanitation and treatment processes. The total number of cells is estimated by counting the air plates. Briefly, the method requires the preparation of a series of decimal dilutions, after which one ml of diluted food is taken and transferred to sterile Petri dishes. (12-15) ml of molten non-selective culture medium is placed in the dish, and circular motions are made by shaking the dish to mix the sample with the culture medium and leave it to solidify and then incubate. At a temperature of 37 for 24 to 48 hours, then the visible colonies (15-300) cells are counted, and then the result is calculated based on the number and dilution factor (Shen& Zhang, 2017).

## 2.13.2.Enterobacteriaceae

The most common genera include *Escherichia*, *Enterobacter*, *Klebsiella Providencia, and Serratia,* which are Gram-negative bacilli found in the intestines of animals and other habitats. *Salmonella, Shigella, Yersinia coli, and E. coli* pathogenic bacteria are the most common gut pathogens. Glucose fermentation, acid generation, and reduce nitrates describe all of its organs, and nitrate reduction is an essential measure of cleanliness and contamination after heat treatment (Robinson,2014). Intestinal bacteria counts are a useful tool for assessing the post-operative environment, such as surfaces in contact with food, and for rapid identification of potential sources of contamination. Because enterococci collectively have a stronger environmental tolerance than coliforms and can better assess glucose-positive and lactose-negative members of dietary bacteria, they may be superior to coliforms as indicators of sanitation. Direct counting is the most common method of detection, which involves the use of solid media containing bile and glucose salts, such as violet-red bile glucose agar (Eden, 2014).

## 2.13.3.Coliform bacteria

Vigorous lactose fermentation at 35 °C for 24-48 hours is one of its gram-negative and facultative anaerobic features (Davidson et al., 2004). *Citrobacter freundii, Enterobacter cloacae, Enterobacter aerogenes, E. coli, and Klebsiella pneumoniae* are all members of the Enterobacteriaceae family. Aeromonas strains from the Aeromonadaceae family, on the other hand, have been identified as coliforms, because of their ability to ferment lactose to form gas and acid within 48 h at 32–37°C (Abbott *et al.*, 2003). Coliform bacteria are used as indicators since their presence in food indicates poor health during the treatment procedure. Special culture media is used to identify these bacteria such as violet-red yellow agar, m-Endo agar/broth, lauryl sulfate, Tryptose broth, brilliant green broth. Most of these media contain lactose as the fermentable basic sugar (Ottosson, 2003).

Membrane filtration is the most common method for decomposing coliforms in low-level polluted water and drinks. The test samples are filtered via a membrane filter with a particular pore size (generally 0.45 mm). The target bacteria grow well when the filter is placed in a sterile Petri dish with adequate medium, but the accompanying spores are repressed at 35°C. Each cell develops into a separate colony that can be counted directly, and the microbial load is estimated as a result. To test the waters, sample amounts of 10, 100, or 250 mL are employed, with the goal of reaching a final colony count range of 20 to 60 cells per filter. There are other testing methods that are focused on the detection of (O'Keefe, 2009).

### 2.13.4.Fecal coliforms/*E. coli*

Fecal coliforms are indicative of fecal contamination often associated with pathogenic bacteria (e.g., *E. coli* O157: H7, *Salmonella*, etc.). The capacity to develop lactose and ferment it at higher temperatures. (45.5°C for food and44.5°C for water during 24–48 h) distinguishes these organisms from the overall coliform group. A typical example of fecal coliforms is *E. coli*. (Venkitanarayanan,2003). *E. coli* serotype O157:H7 produces verocytotoxins, which allow the bacteria to infiltrate and damage gut lining cells, making it infective and virulent even in little levels. When very young children or the elderly get infected, the *E. Coli* O157:H7 serotype is of particular concern, as these populations appear to be at a higher risk of developing severe symptoms (Warriss, 2001). Indicators of fecal contamination should be organisms that are particular to the intestinal system, be present in high numbers in feces to be easily recognized in water/foods after dilution, be able to live at high rates in the test product, and be easily detected, even in very small amounts. Because *E. coli* fits these criteria, it is frequently employed as a fecal contamination indication as well as a hygiene and food safety indicator (Conzelmenn, 1995). Various approaches based on the finding of *E. coli* enzymes have been developed among the alternative testing methodologies. These are based on the usage of 4-methylumbiliferyl-beta-D-glucuronide as a fluorogenic substrate (MUG). This substrate detects b-glucuronidase (GUR) activity produced by the *uid A* gene in *E. coli*. This enzyme metabolizes MUG to release 4-methylumbelliferyl and is found in both liquid and solid media. Fluorescence under long-wavelength UV light yielded these results, and light with a wavelength of 366 nm.

## 2.13.5.Enterococci

Facultative anaerobe, catalase-negative (although some strains are catalase positive in blood agar medium), Gram-positive, oxidase-negative, cocci that normally occur singly, in pairs, or in chains make up the genus Enterococcus. They are all over the place in the environment. primarily being found in the gastrointestinal tracts of humans and animals. They differ from coliforms in that they are salt tolerant and can thrive in a 6.5 percent NaCl solution, as well as being generally resistant to freezing temperatures. Some enterococci species, such as *Enterococcus faecium* and *Enterococcus faecalis*, and are resistant to heat and can survive pasteurization temperatures. Enterococci can establish and remain for lengthy periods of time in food-processing facilities, and they can be monitored primarily as indications of process hygiene or food quality. This indicator may be useful in identifying poor manufacturing procedures in specific circumstances. They are frequently viewed as being more dependable than coliform count as an indication of sanitary quality since they are better able to survive in salted food. In addition, the official benchmark for establishing the quality of potable and drinking water is the absence or presence of enterococci. Many different media, including as m-Enterococcus Agar, KF Streptococcus Agar, Citrate Azide Tween Carbonate (CATC) Agar, Kanamycin Aesculin (Esculine) Azide (KAA) Agar, and Enterococcal Chromocult Agar, can be used to hypothetically enumerate and isolate enterococci from a variety of meals. 5-10 model colonies are isolated and subjected to traditional biochemical assays or fast identification technologies to confirm the probable positive results.

**Table (2.3) 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 - $<10^2$ | $> 10^2$ |

When the testing capability for this criteria is ready, it will be applied.Notes:

a. Heat-treated foods, shellfish, and cheeses all fall within the Enterobacteriaceae criterion (with the exception of cheeses ripened using a Hafnia alvei or Proteus vulgaris culture). It does not apply to fresh fruits and vegetables, or foods that incorporate fresh fruits and vegetables as ingredients, such as sandwiches with salad, because these items can contain high levels of Enterobacteriaceae as part of their normal microflora.

b. Raw milk cheeses are exempt from the criteria.

# *Chapter Three*

# *Materials and Methods*

# 1Materials and methods:

## 3.1-Materials

### 3.1.1.Equipment and Instruments

The following equipment and apparatus have been utilized throughout the study, as shown in **Table ( 3-1 ).**

**Table(3-1): equipment, Instruments, and producers used in this study**

| Equipment/Instruments | Company | Origin |
|---|---|---|
| Aerobic incubator | Memmert | Germany |
| Autoclave | Hirayama | Japan |
| Benson burner | Membrane | Germany |
| Centrifuge | Hermle | |
| Compound Light Microscope | Zeiss | |
| Conical flask (different size) | Himedia | India |
| Digital camera | Sony | Japan |
| Eppendorf Centrifuge | Hettich | (Germany) |
| Forceps | Himedia | India |
| Gel Electrophoresis System | Cleaver Scientific | (UK) |
| Laminar flow cabinet | Labogene | Denmark |
| Micropipettes (different volume) | Eppendorf | Germany |
| Microwave Oven | LG | Korea |
| PCR Thermal Cycler | Techne | UK) |
| Platinum wire loop | Himedia | India |
| Refrigerator | Concord | Germany |
| Sensitive electronic balance | Kern | USA |
| UV- transilluminator | Cleaver | England |
| Vortex | Gemmy | Taiwan |
| VITEK-2 compact system | BioMérieux | France |
| Water bath | Kottermann | Germany |
| Volumetric cylinder | HAD | China |

### 3.1.2.Disposable material

The following disposable material has been used in this study, as shown in **Table (3-2).**

**Table(3-2): Disposable material used and their Manufacturers**

| **Disposable** | **Manufacture** | **(Origin)** |
|---|---|---|
| Coton | | Chine |
| Cotton swap | Afco | Jorden |
| Dropper | HAD | China |
| Eppendorf rack | HAD | |
| Eppendorf tube | Sigma | England |
| Glass slides | Himedia | India |
| Latex Gloves | Broche | Malaysia |
| Mask | | China |
| Micropipette tips | Afco | Jordan |
| Parafilm | | |
| PCR tubes 50µl | Eppendorf | Oxford |
| Petri dishes | Afco | Jordan |
| Plastic test tubes 10 ml | | |
| Syringes | | Chain |
| Test tube rack | Lap Tech | India |
| Volumetric cylinder | HAD | China |

## 3.1.3-Culture media

The culture media used in this investigation were sterilized by autoclaving at 121 ° C for 15 minutes, as per the manufacturer's recommendations **Table(3-3).**

**Table (3-3) : The media utilized in this study.**

| Media | Purpose | Prepare method | Company and country of origin |
|---|---|---|---|
| Blood Agar | It can be used to determine an organism's hemolytic capabilities.(Moha&Lehman, 2019). | 40g/L and add 5% of sheep blood | |

| | | | |
|---|---|---|---|
| Brain Heart Infusion Broth (BHI) | It is a highly nutritious general-purpose growth medium recommended for preservation. It activates bacterial growth and keeps in frozen by adding15% glycerol (Prajapati et al.,2017). | 37.5 g/L | Himedia (India) |
| Eosin methylene blue (EMB) | EMB is the selective and differential medium for coliforms. | 36 g /L | |
| Luria Broth (LB) | It is used extensively in recombinant DNA work and other molecular biology procedures | 20 g /L | |
| MacConkey agar | Iit is used to isolate Gram-negative and enteric | 52 g/L | |
| Mannitol Salt Agar | It was previously utilized to isolate and distinguish S. aureus from a beef sample.(Cappuccino and Welsh, 2020). | 111 g/ L | |
| Mueller Hinton Agar | It is used to determine the sensitivity of bacteria to antibiotics (CSLI,2019) | 38g/L | |
| Mueller Hinton Broth | It is used for antibiotic sensitivity testing for bacteria (Cappuccino and Welsh, 2020) | 19 g/L | |
| Nutrient Agar | A basic culture medium that can grow a broad range of bacteria and is commonly used in microbiological tests. It is one of the non-selective medium used in ordinary microorganism production. (Cappuccino& Welsh,2020). | 28 g/L | |

| | | | |
|---|---|---|---|
| Nutrient Broth | It is a general-purpose medium that can be used for the cultivation of a non-highly nutritious bacteria(Cappuccino& Welsh,2020). | 38 g/L | |

### 3.1.4. Chemical and biological material

The biological and chemical materials used in this study are listed in **Table (3-4).**

**Table (3-4) the chemical and biological material utilized in this study.**

| Chemicals | Company and country of origin |
|---|---|
| Agarose, TBE buffer | Condalab (Spain) |
| Alcohol 75% | Al-Joud (Iraq) |
| Barium Chloride | BHD (England) |
| Catalase reagent | Himedia (India ) |
| DNA Loading dye | Promega(USA) |
| Ethanol (95%) | BHD (England) |
| Glycerol | |
| Normal Saline | Mehico (India) |
| Nuclease Free Water | Bioneer (Korea) |
| Oxidase reagent | Himedia (India ) |
| Red safe | Bioneer (Korea) |
| Sulfuric acid ($H_2SO_4$) | BHD (England) |

## 3.1.5. Diagnostic kits

The commercial kits used in the present study are illustrated in **table (3-5)**.

**Table(3-5): Type of Kits used in the study.**

| Kits | Components of the kit | Company(origin) |
|---|---|---|
| Gram stain | 1-Crystal violet Solution<br>2-Iodine Solution<br>3-Decolorizer Solution | SyrBio (Syria)<br>Sigma/ Germany |

| | | |
|---|---|---|
| | 4-Safranin Solution | |
| DNA Extraction Kit | 1-Solution DS (15 ml )<br>2-Solution MS( 20 ml )<br>3-Proteinase K( 20 mg/ml )<br>4-Wash BUFFER PS<br>5- TE Elution Buffer (10 mM Tris–HCL, 1 mM EDTA, PH 8.5) | Favorgen/ Taiwan |
| DNA ladder 100bp | A ladder consists 1-double-stranded DNA<br>2-Loading dye has a composition (15% Ficoll, 0.03% bromophenol blue, 0.03% xylene cyanol, 0.4% orangeG, 10 Mm.tris-HCl, pH 7.5 and 50 mM EDTA). | Bioneer(Korea) |

## 3.1.6. Antibiotic discs

**Table (3-6) :the antibiotic discs use in this study.**

| Antibiotic | Potency(Disc load (µg)) | Company(Origin) |
|---|---|---|
| Oxacillin | 5 | Bioanalyse (Turkey) |
| Chloramphenicol | 30 | |
| Ciprofloxacin | 10 | |
| Levofloxacin | 5 | |
| Clindamycin | 15 | Biolab(Hungary) |
| Erythromycin | 15 | |
| Gentamicin | 10 | |
| Nitrofurantoin | 300 | |
| Penicillin G | 10 | Condalab (Spain) |
| Rifampicin | 5 | Biolab (Hungary) |
| Tetracycline | 10 | |
| Trimethoprim | 10 | |

**Table:(3-7)primer pairs ,sequences, and Amplicon Size of Phylogenetic typing**

| Gene | **Primer sequence (5-3)** | **Size (bp)** | **References** |
|---|---|---|---|
| ***16S-RNA* Species specific** | F-**TATGGAGGAACACCAGTGGCGAAG** | **791** | Mori *et al.*,*(*2014) |
| | R-**TATGGAGGAACACCAGTGGCGAAG** | | |
| ***sea*** | F-**CCTTTGGAAACGGTTAAAACG** | **127** | Chapaval *et al* ., (2006) |
| | R-**TCTGAACCTTCCCATCAAAAAC** | | |
| ***seb*** | F-**TCGCATCAAACTGACAAACG** | **477** | Salasia *et al.*, 2004 |
| | R-**GCAGGTACTCTATAAGTGCC** | | |
| ***sec*** | F-**CTCAAGAACTAGACATAAAAGCTAGG** | **271** | Salasia *et al.*, 2004 |
| | R-**TCAAAATCGGATTAACATTATCC** | | |
| ***sed*** | F-**CTAGTTTGGTAATATCTCCTTTAAACG** | **319** | Salasia *et al.*, 2004 |
| | R-**TTAATGCTATATCTTATAGGGTAAACATC** | | |
| ***see*** | F-**CAGTACCTATAGATAAAGTTAAAACAAGC** | **178** | Chapaval *et al* ., (2006) |
| | R-**TAACTTACCGTGGACCCTTC** | | |

## 3.1.8.Solutions

### 3.1.8.1. McFarland standard solution

To make the 0.5 McFarland's standard tube ($1.5\times10^8$ CFU/ml), a barium sulfate precipitate was made by mixing 0.5 ml of 1.175 percent barium chloride with 1 percent sulfuric acid. The solution was used to visually evaluate the turbidity of a bacterium suspension to that of a 0.5 McFarland's standard (Eduardo *et al*.,2018; Jain *et al*., 2020).

### 3.1.8.2.Tris-Borate-EDTA (TBE buffer)

A buffer of 1X TBE was prepared from of 10X TBE buffer. To give the final concentration, 1X TBE added 100ml of 10X TBE to 900ml of sterile distal water. This solution was used to dissolving agarose and in electrophoresis (Heintz and Gong, 2019).

### 3.1.8.3. TE-buffer

TE buffer (Tris–HCl (10 mM) ,100Mm NaCl and EDTA (1 mM), pH 8.0). Used in DNA extraction (Cheng and Jiang, 2006).

## 3.1.9. Reagents

### 3.1.9.1. Oxidase reagent

It is made by dissolving one gram of tetra methyl-P-phenyl diamine dihydrochloride in 100 ml of distilled water and then adding another 100 ml of distilled water. D.W. investigated the capacity of bacteria to produce the oxidase enzyme. (Cappuccino and Welsh , 2020).

### 3.1.9.2. Catalase reagent

To study bacteria's ability to create the enzyme catalyzes analyzed for hydrogen peroxide, hydrogen peroxide was produced at a concentration of 3% (Cappuccino and Welsh, 2020).

## 3.2. Methods

### 3.2.1. Study design

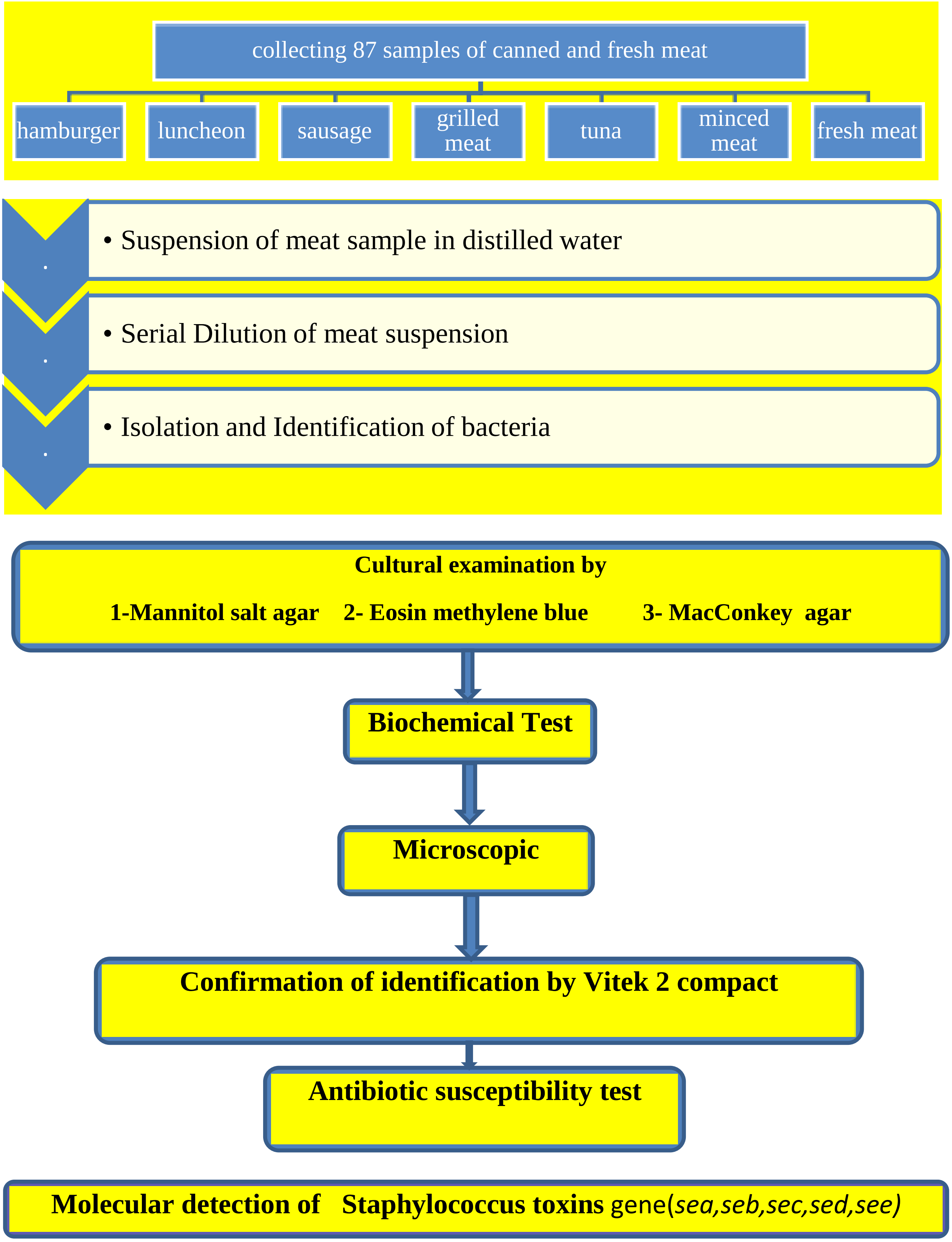

**Diagram (3-1): Flow chart illustrates the study design**

## 3.2.2. Sample collection

A total of 87 meat samples were gathered, with 31% being fresh meat and 68.96% being canned meat (imported and local). (Appendix 1). These samples were gathered in dry and sterile condition at random from several supermarkets in Babylon, Iraq, at the period from November 2021 and January 2022. One gram of each sample was crushed, mixed with 10 ml of distilled water, and a series of dilutions were made, then the last two dilutions were taken and poured onto Nutrient Agar in a sterile Petri dishes. To mix the sample with the medium, the plates were moved clockwise and anticlockwise several times. It was allowed to solidify before being incubated, and incubated for( 24-48 ) hours at 37 °C (Samaha *et al*., 2012; Khalafallah *et al*.,2020).

## 3.2.3. Counting of bacterial species in food samples

After incubation of plates inverted from at 37 °C for 24–48 hours by spreading methods, and manually count colonies on the agar plates. (APHA,2001). Counting of microbial colonies at (30 -300) was measured. The CFU /ml was measured by the following equation:

CFU/ml = (number of colonies * dilution factor) / volume spread on plate

## 3.2.4. Identification of bacterial isolates

### 3.2.4.1. Microscopic examination

The gram stain reaction was performed by placing a drop of water on a microscope slide. A small part of bacterial colony was then transferred, mixed with distilled water, and smeared using a sterile cold loop. The slide was air dried and then heat fixed, by passing the slider briefly through the burner flame several times. Then, the slide was stained with Gram stain and examined under an oil-immersion lens (Rahimzadeh *et al.,* 2017).

### 3.2.4.2 .Cultivation of bacteria on differential media

The bacteria were cultured on differential culture media : mannitol salt agar, eosin methylene blue ,and MacConkey agar. The quantities specified by the factory were measured and placed in a sterilizer, then poured into petri dishes and left to solidify, then bacteria were cultured by streaking method on petri dishes and incubated at 37°C for 24 hr.

### 3.2.4.3.Vitek 2 compact system

To confirm suspected isolates, the Vitek 2 compact technique is employed. The VITEK 2 is a growth-based microbiology device that is fully automated. Each of the 64 wells on the reagent cards can accommodate a different test substrate. In the presence of inhibitory chemicals, substrates measure numerous metabolic processes such as enzyme hydrolysis, alkalinization, growth and acidification. On both sides of the card, an optically transparent layer allows for the required degree of oxygen transfer while maintaining a sealed vessel that prohibits contact with the organism-substrate admixtures. A pre-inserted transfer tube for inoculation is included with each card. Bar codes on the cards carry information on the product kind, lot number, expiration date, and a unique identifier that may be linked to the sample before or after it is loaded.

### 3.2.4.4.Biochemical tests

#### 3.2.4.4.1.Catalase test

A few drops of 3% $H_2O_2$ were added to colonies of bacteria transferred from cultivation by wooden stick to a slide. The appearance of gas bubbles has been identified as a positive test (Cappuccino and Welsh, 2017).

### 3.2.4.4.2. Oxidase test

The filter paper is moistened with about four drops of tetra methyl-P-phenyl diamine dihydrochloride, then transferred by a ring of bacterial colonies, mixed, and then left for ten second. If the color changes to dark blue or black, the result is positive, but if it does not change, the result is negative (Cappuccino and Welsh, 2017).

### 3.2.4.4.3 Blood hemolysis test

Medium blood agar was prepared according to the manufacturer's instructions, and after sterilization blood agar base by autoclaving, temperature was reduced to 40 degrees, then blood was added to it in a ratio of( 5%), then poured into dishes and allowed to solidify. Then they were inoculated with colonies of bacteria and incubated for 24 hours at 37°C. A halo of hydrolysis surrounding colonies suggests a positive result (Mohan and Lehman, 2019).

### 3.2.4.4.4. Mannitol fermentation test

This assay was performed by inoculating each Gram-positive bacterial isolate with mannitol salt agar and incubating at 37°C for 24 hours. The sample was positive if the color changed to yellow (Cappuccino and Welsh, 2017).

### 3.2.5. Maintenance of bacterial isolates

### 3.2.5.1. Short - term storage

The separated bacteria were preserved by streaking them on brain heart infusion agar medium, then incubating them at 37° C for 24 hours to get a lot of growth, and finally stored them at 4° C for a few weeks (Jain *et al.,* 2020).

### 3.2.5.2. Long -term storage

The bacteria can be stored in a screw-capped tube at -20 °C for a long time in Brain Heart Infusion Broth with 15% glycerol (Jain *et al.,* 2020).

## 3.2.6. Antibiotics susceptibility test

Several antibiotics disk for antibiotic susceptibility test by Kirby-Bauer (Kirby *et al* ., 1966) An isolated colony of the tested bacteria was inoculated into three milliliters of Mueller-Hinton broth and incubated at 37°C for 24 hours. to match 1.5 x $10^8$ CFU/ml McFarland tube concentration, 3 mL of normal saline and the produced bacterial drops in Mueller-Hinton broth. The Mueller-Hinton agar medium's surface is inoculated with 0.1 ml of the bacterial culture, and let it to dry. A maximum of five antibiotic tablets were placed on the dish. Five discs can be evenly spaced around 15 mm from the plate's edge, with one disc in the plate's center. The plates were incubated for (18-24) hours at 37°C. (Kirby-Bauer *et al.,* 1966). The inhibition zones that resulted were measured using a ruler and compared to the inhibition zones that were obtained by (CLSI, 2021).

## 3.2.7. DNA extraction

Staphylococci were incubated in Luria broth and incubated at 37 °C for 24 h, then 1 mL of cell suspension was transferred to a 1.5 mL Eppendorf tube for bacterial DNA extraction by silica based membrane technology DNA extraction method including the following steps:

1-Centrifuged 1 ml of cell suspension for 1 min at 12000 rpm.

2- After removing the supernatant, add 108 µl DS solution and 72 µl lysyme . Mix immediately by brief vortex, than incubated at 37 °C for 30 min

3-Add 20 µl Proteinase K , mix thoroughly by brief vortex , then incubated at 55 °C for 30 min

4- Add 220 µl Solution MS, mix thoroughly by brief vortex ,then incubated at 65 °C for 10 min

5- Add 220 µl ethanol (96-100%)to the lysate , and mix thoroughly by brief vortex

6- Then the mixture is withdrawn by a pipette and transferred to a spin column of a volume of two ml, then placed in centrifuge at 12000 rpm for 1 min. discard flow – through

7- Add 500 µl wash buffer PS ,and centrifuge for 1 min at 12,000 rpm . discard flow – through

8- Add 500 wash buffer PE ,and centrifuge for 1 min at 12,000 rpm . discard flow –through . Repeat this step again

9- Centrifuge for 3 min at 12,000 rpm to dry the column membrane , discard flow – through and collection tube .

10 – Place the spin column in a clean 1.5 ml microcentrifuge tube and pipet 30 -100 µl Elution Buffer TE directly onto the membrane . Incubate at room temperature for 2 min

11- Centrifuge for 2 min at 12000 rpm . then store the DNA at -20 °C

## 3.2.8. Polymerase chain reaction

### 3.2.8.1. Thermal cycling conditions

To make a huge number of copies of a gene, the Polymerase Chain Reaction is used. Otherwise, the amount of DNA available is restricted and cannot be used with other types of analysis. The PCR reaction is carried out using an automated cycler that quickly warms and cools the tubes using the reaction mixture. For 35 cycles, PCR was performed in three primary phases: 1) denaturation, 2) annealing, and 3) extension (Mohan and Lehman, 2019).

### 3.2.8.2. **Preparation of primers**

The (F, R primer) used in this study, dissolved in 300 µL for the other ***S. aureus*** toxins primer according to the manufacturers instructors to give a final concentration of 100 µL and stored in the deep freezer until used in PCR amplification. Then, take 10 µL from each forward and reverse primer and in the new tube and add 180 µL of nuclease-free water to obtain a final volume 200 µL and the concentration 10 ml/ µL

**Table(3-8) : Contents of the reaction mixture of PCR**

| **NO.** | **Contents of the reaction mixture** | | **Volume** |
|---|---|---|---|
| **1** | **master mix** | | **12.5µl** |
| **2** | **Primer** | **forward** | **2 µl** |
| **3** | | **Reverse** | **2 µl** |
| **4** | **DNA template** | | **4µl** |
| **5** | **Nuclease free water** | | **4.5µl** |
| **Total volume** | | | **25µl** |

**3.2.8.2. PCR Thermal cycling conditions for the detection of different gene in *Staphylococcus aureus***

**Table (3-9):Primer for characterization**

| **Primer** | **Conditions** | **Reference** |
|---|---|---|
| ***16SrRNA Species specific*** | Step 1: 95.0ºC, 2 min.<br>Step 2: 95.0ºC, 30 sec.<br>Step 3: 63.9ºC, 30 sec.<br>Step 4: 72.0ºC, 20.0 sec.<br>Step 5: Repeat steps 2-4 29 more times<br>Step 6: 72.0ºC, 5 min.<br>Step 7: 4.0ºC, forever | Mori *et al.*,2014 |
| ***Sea*** | Step 1: 95.0ºC, 2 min.<br>Step 2: 95.0ºC, 30 sec.<br>Step 3: 56.0ºC, 30 sec.<br>Step 4: 72.0ºC, 20.0 sec.<br>Step 5: Repeat steps 2-4 29 more times<br>Step 6: 72.0ºC, 5 min.<br>Step 7: 4.0ºC, forever | **Haghi *et al.*,2021** |
| ***Seb*** | Step 1: 95ºC, 2 min.<br>Step 2: 95ºC, 30 sec.<br>Step 3: 56.2ºC, 30 sec.<br>Step 4: 72ºC, 50.0 sec.<br>Step 5: Repeat steps 2-4 29 more times<br>Step 6: 72ºC, 5 min.<br>Step 7: 4ºC, forever | Haghi *et al.*,2021 |
| ***Sec*** | Step 1: 95ºC, 2 min.<br>Step 2: 95ºC, 30 sec.<br>Step 3: 59.1ºC decrease 0.5ºC per cycle, 30 sec.<br>Step 4: 72ºC, 30.0 sec.<br>Step 5: Repeat steps 2-4 14 more times<br>Step 6: 95ºC, 30 sec.<br>Step 7: 52.1ºC, 30 sec.<br>Step 8: 72ºC, 30.0 sec.<br>Step 9: Repeat steps 6-8 19 more times<br>Step 10: 72ºC, 5 min.<br>Step 11: 4ºC, forever | Haghi *et al.*,2021 |

| | | |
|---|---|---|
| ***Sed*** | Step 1: 95.0°C, 2 min.<br>Step 2: 95.0°C, 30 sec.<br>Step 3: 57.8°C, 30 sec.<br>Step 4: 72.0°C, 40.0 sec.<br>Step 5: Repeat steps 2-4 29 more times<br>Step 6: 72.0°C, 5 min.<br>Step 7: 4.0°C, forever | Haghi *et al.*,2021 |
| ***See*** | Step 1: 95.0°C, 2 min.<br>Step 2: 95.0°C, 30 sec.<br>Step 3: 58.0°C, 30 sec.<br>Step 4: 72.0°C, 20.0 sec.<br>Step 5: Repeat steps 2-4 29 more times<br>Step 6: 72.0°C, 5 min.<br>Step 7: 4°.0C, forever | **Haghi *et al.*,2021** |

## 3.2.9. Detection of amplified products by agarose gel electrophoresis:

### 3.2.9.1.Agarose gel preparation:

The PCR product was confirmed by agarose gel electrophoresis. Separating DNA fragments on an agarose gel electrophoresis is the most successful method. The amount of agarose in a gel varies depending on the size of the DNA fragments to be separated, ranging from 0.5 percent to 2%. After extraction, a 0.7 percent gel was utilized to get good separation of genomic DNA (5-10 kb), whereas 1.5 percent -2 percent gels were used to get high resolution for tiny PCR product fragments (0.2-1 kb). However, in 100ml of 1TBE buffer, the specified weight of agarose was added and melted in the microwave until the solution became clear. To reach a final concentration of 0.5g/ml, the Agarose was cooled to 50-55°C and 5l of simply safe dye (10 mg/ml) was added to 100 ml of melting Agarose gel. The Agarose was put into the gel tray, sealed at the ends, and the comb was appropriately inserted before drying. Each sample was placed into its own well of the gel, with a marker in one of them. (The length of agarose gel electrophoresis is 45 minutes for genomic DNA and 1 hour and 30 minutes for PCR product.)

Electrodes were connected appropriately, and the run was applied according to the gel percentage and size of the gel (Sambrook and Russel, 2006;Lee *et al*., 2012;Cappuccino and Welsh, 2020).

1. Check that the teeth of the comb are roughly 0.5mm above the gel bottom by placing the gel-casting tray in a plastic tray.
2. Prepare 500ml of TBE (1X) by combining 50ml of TBE (10X) stock solution with 500ml deionized water.

3. In a 500ml flask, pour 100ml of the buffer and 0.8g of Agarose. Melt the Agarose by heating the solution on a hot plate for about 10 minutes (microwave). Swirl the Agarose solution gently to ensure that the Agarose is completely dissolved, with no visible Agarose particles.

4. Cool the Agarose solution to around 50°C before adding 2-3 µl of Simple safe stock solution. Pour the Agarose into the gel casting tray in a slow, steady stream. Using the yellow tip, remove any air bubbles.

5. Place the comb roughly 1.5cm from the gel's edge. Allow for 20–30 minutes for the Agarose to harden. Remove the comb with a gently back and forth motion when the Agarose has set, being careful not to break the gel.

6. Remove the gel-casting tray and set it on the gel box's central supporting platform.

7. Fill the buffer chamber with electrophoresis buffer until it reaches a level of 0.5–1cm above the gel's surface.

8. Using a yellow tip, load the samples into the wells. Just above the well, place the tip under the surface of the electrophoresis buffer. Remove the sample.

gently, allowing it to drop to the well's bottom make sure that the sample does not spill into a nearby well. Samples must be placed into sample wells in a certain order. It is best to leave the wells closest to the edge of the gel unfilled when loading fewer samples than the number of wells.

9. Fill one well with 20 of DNA specimen and 5 µl of ladder molecular weight marker on either side of the gel (flanking the sample line).

10. Connect the electrodes to the lid of the gel box. The DNA will migrate away from the well to the positive (red) electrode. Activate the power supply.

11. Continue electrophoresis until the tracking dye has moved a minimum of 10 cm along the length of the gel.

### 3.2.9.2. Reading the electrophoresis results

The electrophoresis findings are detected with the use of a gel documentation system. The base pair of DNA determined by the ladder. UV transilluminator used for the visualization of DNA bands and optical camera used for gel photography. It was assured that all PCR-resolved bands were specific and consisting of just one transparent and sharp band to be effectively sequenced (Mohan and Lehman, 2019).

### 3.2.9.3.Statistical analysis

Statistical analysis data was calculated by using SPSS software version 16.0. (SPSS, Chicago, IL), $p < 0.05$ considered statistically significant, while $p >= 0.05$ was not statistically significant.

# *Chapter Four*

# *Results and Discussion*

# 4.Results and Discussion:

## 4.1. Bacterial identification and counting

Eighty Seven meat samples were randomly collected from different markets in Hilla city in Iraq. These samples were distributed as following :27 samples of fresh meat from butchers, 60 canned samples included ( luncheon meat and chicken 15 (25%), sausage 10 (16.6%), tuna 9 (15%), chicken pieces 9 (15%) Minced meat 7 (11.6%), grilled meat 5 (8.3%), hamburger 5 (8.3%),(Appendices 1). Samples were collected during the period from November 2021 to January 2022 using a sterile, clean and dry container. The distribution of canned samples .

The following Table(4-1) shows the number of bacteria isolated for each sample

**Table (4-1) : Number of bacteria isolated for each samples from meat**

| Samples | No. of bacteria |
|---|---|
| **Luncheon** | **15(17.2%)** |
| **Sausage** | **10(11.2%)** |
| **Tuna** | **9(10.3%)** |
| **Chicken pieces** | **9(10.3%)** |
| **Minced meat** | **7(8%)** |
| **Grilled meat** | **5(5.8%)** |
| **Hamburger** | **5(5.8%)** |
| **Fresh meat** | **20(22.9%)** |
| **No growth** | **7(8%)** |
| **Total** | **87** |

For isolation and initial identification, standard microbiological procedures were used, such as culture in differential media such as mannitol salt agar, EMB, MacConkey agar, gram stain reaction , and biochemical tests (catalase and oxidase). The highest bacterial count was recorded with $4.08 \times 10^8$ CFU\g from Tazaj beef luncheon samples, the lowest number was in fresh meat $1\times10^8$.

The table (4-2) shows large differences in the standard deviation between the isolates, and this is because there are samples that have a very high contamination rate and others are less contaminated, and this is due to several reasons, including that there is a section of canned goods that moved between several countries before settling in supermarkets, for example, they were made in a country and then exported to another country and then transferred to Iraq. Among the other reasons is the shelf life, as the shelf life was different between products, and the storage of these cans differed between kept in the refrigerator or on shelves where the varying temperatures. In addition to the effect of the type of packaging used in canning on the growth of bacteria, such as rip-off bags or openable cans.

**Table (4-2): Mean of bacterial total count ±SE of the meat and meat product samples :**

| samples | Mean ±SE |
|---|---|
| Luncheon | $73\times10^8\pm21.4$ |
| Sausage | $55\times10^8\pm31.4$ |
| Tuna | $142\times10^8\pm63.2$ |
| Chicken pieces | $97\times10^8\pm59.5$ |
| Minced meat | $66\times10^8\pm9.1$ |
| Grilled meat | $36\times10^8\pm9.7$ |
| Hamburger | $45\times10^8\pm12.8$ |
| Fresh meat | $5\times10^8\pm1.1$ |

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 20-300 colonies per plate should be used (Artés *et al.*,2007) ,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 to grow the 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 freezing and thawing the products and re-freezing 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 day.

Chapman agar, also known as mannitol salt agar, is a selective medium for isolating, counting, and distinguishing Staphylococcus from clinical, food, and cosmetic samples. This is a selective as well as differential agar. The medium will select organisms that can thrive in.

high-salt environments .(sodium chloride), and the fermentation of mannitol, as seen by the yellowing of the pH indicator (phenol red), allows for diagnosis guidance. This medium's selectivity is due to the inclusion of sodium chloride (7.5%), which obstructs most Gram positive and Gram negative microorganisms.

When an organism ferments mannitol, an acidic byproduct is produced, turning the phenol red in the agar yellow. It is used to isolate presumptive pathogenic (pp) Staphylococcus species selectively. Other coagulase-negative staphylococci produce little pink or red colonies that do not modify the color of the medium, but *Staphylococcus aureus* develops yellow colonies with yellow zones

Samples were also cultured on MacConkey agar and to distinguish Gram-negative bacteria **.**Then the bacteria grown on MacConkey's medium were cultured on Eosin methylene blue (EMB), a facultative dye for gram-negative bacteria. It contains a dye that is poisonous to gram-positive bacteria as well as bile salts that are harmful to gram-negative bacteria other than coliform bacteria. Eosin methylene blue is facultative and media-differentiated for coliforms. It is a mixture of two dyes, eosin and methylene blue in a ratio of 1:6. One of the most popular applications of the dye is in the preparation of eosin and methylene blue agar, differential microorganisms that slightly inhibit the growth of Gram-positive bacteria and support the color reagent that distinguishes lactose-fermenting organisms Metal (*Escherichia coli)* and that do not ferment (such as Salmonella Shigella), organisms that ferment lactose exhibit "nuclear colonies"—colonies with a dark center .

Bacteria were cultured in the medium of Blood agar which is used to detect cytolytic toxins that cause hemolysis (the destruction of RBCs).

The present study revealed ratio of sample produced β-hemolysin was 27 (33.75%) .

According to the diagnosis of the Vitek device (Appendix 2) , the number of bacteria was as shown in the following table:

During the diagnosis with the Vitik, the types of isolated bacteria were determined, and they are shown in the Table (4-3)

**Table(4-3) : Number of bacteria according to the diagnosis of the food sample.**

| No. | Samples | Staphylococcus | Enterobacteriaceae | different bacteria |
|---|---|---|---|---|
| 1 | luncheon | 8 | 6 | 1 |
| 2 | Sausage | 4 | 5 | 1 |
| 3 | Tuna | 4 | 3 | 2 |
| 4 | chicken pieces | 4 | 4 | 1 |
| 5 | Minced meat | 4 | 2 | 1 |
| 6 | grilled meat | 2 | 2 | 1 |
| 7 | hamburger | 2 | 2 | 1 |
| 8 | fresh meat | 12 | 6 | 2 |
| Total | | 40 | 30 | 10 |

The largest proportion was bacteria *S. aureus* As its percentage of the total number of isolated bacteria is **(55%** ) from the total isolates of Staphylococcus spp. and **(27.5 %)** from the total number of isolates . as shown in Table (4- 5 ).

The prevalence of *S. aureus* was closer to the present study which has 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 ,and higher than (Omwenga, 2022) who found the prevalence of *S. aureus* was (20.3%).

In the table (4-6) explain the Number and percentage of *S. aureus* isolated from different meat sample.

**Table(4-4) :Explains the types of bacteria according to Vitek device and their numbers**

| **Staphylococcus** | **Enterobacteriaceae** | **Different bacteriaceae** |
|---|---|---|
| ***Staphylococcus aureus*** (n=22) | ***Enterobacter cloacae***(n=18) | ***Burkholderia cepacia***(n=1) |
| ***Staphylococcus lentus***(n=18) | ***Klebsiella pneumonia***(n=12) | ***Streptcoccus agalactiae***(n=3) |
| ------------------------- | --------------------------- | ***Kocuria kristinae***(n=1) |
| ------------------------- | ---------------------------- | ***Micrococcus luteus*** (n=4) |
| ------------------------ | -------------------------- | ***Gemella sanguinis(n=1)*** |

**Table (4-5 ): Number and percentage of *Staphylococcus aureus* isolated from different meat samples (n=22)**

| No | Sample | No. | Percentage |
|---|---|---|---|
| 1 | Luncheon | 5 | 22.72% |
| 2 | Sausage | 2 | 9.09% |
| 3 | Tuna | 3 | 13.63% |
| 4 | chicken pieces | 2 | 9.09% |
| 5 | Minced meat | 3 | 13.63% |
| 6 | grilled meat | 2 | 13.63% |
| 7 | Hamburger | 1 | 4.54% |
| 8 | fresh meat | 4 | 18.18% |

The largest percentage of *S. aureus* bacteria in microscopic examination was 22 isolates.

As a result of the biochemical tests and culture on the media, the percentage of Staphylococcus bacteria was 40 samples (50%), and Enterobacteriaceae bacteria 30 samples (37.5%), 10 samples(12.5%) represent of different bacteria, these results were confirmed by using Vitik device. The prevalence of bacterial isolates is shown in Figure (4-6)

## 4.3. Antibiotic susceptibility test for *S. aureus:*

Antibiotic susceptibility testing was performed using 12 types of antibiotics mentioned in the[( 3.1..6.)] for 22 sample from *S. aureus*. The results shows that the highest resistance was against Trimethoprim 22(TMP)100% and a penicillin G (P)22(100%), followed by Erythromycin (E)19**(86.6%)**,Clindamycin (AD) 19**(86.6%)**,chloramphenicol (C) 17(**77.27** %) , tetracycline (TE) 15(68.18%) , Oxacillin (OX) 14(80%), the lowest resistance against rifampicin (RA)3 (13.63%), and gentamicin

(CN)2(9.09%), and no isolates found resistant to Nitrofurantoin, Ciprofloxacin, and Levofloxacin which make them a good choice for treatment Table (4-6) .

**Table (4-6): Antibiotics susceptibility test of *S. aureus* isolated from meat and meat product.**

| Antibiotic | Potency (Disc load (µg)) | Resistant | Sensitive |
|---|---|---|---|
| **Trimethoprim(TMP)** | 10 | **(100%)** | **-----** |
| **Penicillin G (P)** | 10 | **(100%)** | **-----** |
| **Erythromycin** | 15 | **(86.6%)** | **(13.6%)** |
| **Clindamycin(DA)** | 15 | **(86.6%)** | **(13.6%)** |
| **Chloramphenicol ( C)** | 30 | **(77.27%)** | **(22.27%)** |
| **Tetracycline(TE)** | 10 | **(68.18%)** | **(31.81%)** |
| **Oxacillin(OX)** | 5 | **(63.63%)** | **(36.36%)** |
| **Rifampicin(RA)** | 5 | **(13.63%)** | **(86.36%)** |
| **Gentamicin (CN)** | 10 | **(9.09%)** | **(90.9%)** |
| **Nitrofurantoin(F)** | 300 | **----** | **(100%)** |
| **Levofloxacin(LEV)** | 5 | **----** | **(100%)** |
| **Ciprofloxacin(CIP)** | 10 | **----** | **(100%)** |

From the table, It has been found that Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria , and this agrees with each of the following research (Zhou, 2021; Breijyeh *et al.*,2020), Because of the increased protection provided by the outer membrane, Gram-negative bacteria are more resistant to antimicrobial agents than Gram-positive bacteria. The Methicillin Resistant *S. aureus* is identified utilizing the Oxacillin disc antibiotic, according to( Palavecino ,2020).

The isolated bacteria were highly resistant to, Trimethoprim(TMP)and Penicillin G (P) used in this study. This result was agree with (Yousif *et al.*, 2022) who found all isolates resistance to penicillin. and (El-Tawab *et al.*,2022) who found all isolates resistance to penicillin and Trimethoprim.

The most resistant bacteria isolated to Trimethoprim antibiotic used in the current study are related to the following resistance mechanism Trimethoprim inhibits the production of tetrahydrofolate, the active form of folic acid, which has antimicrobial properties, by organisms that are vulnerable Exogenous foliate cannot pass through bacterial cell walls. There have been several methods of bacterial resistance to trimethoprim reported. Cell wall permeability factors that prevent the medication from binding to dihydrofolate reductase have been blamed for intrinsic resistance, as it occurs with *Pseudomonas aeruginosa*, or the existence of a dihydrofolate reductase resistant to trimethoprim inhibition, as it occurs with *Neisseria garosenes*, *Clostridium perfringens*, and *Bacteroides fragilis*. Susceptible strains can gain resistance over time. This is caused by a chromosomal mutation that causes the development of a dihydrofolate reductase, which has different degrees of activity, on the other hand, is less susceptible to trimethoprim inhibition. Resistance is less typically transmitted by R factors or plasmids. Trimethoprim resistance is extremely high thanks to these extrachromosomal DNA fragments. (MIC > 1,000 µg/ml) by encoding the synthesis of a dihydrofolate reductase unaffected by the chemical. Transposons, DNA segments that may “jump” from one replican to the next, provide resistance (as from plasmid to chromosome), The emergence during therapy of mutants that requires exogenous thymine for DNA synthesis is also an intriguing unusual type of acquired resistance to trimethoprim. As a result, the drug’s deadly metabolic lesion is avoided. These thymine-dependent strains have been found most commonly in individuals with renal calculi who had been treated with trimethoprim-suIFamethoxazole for a long time. Patients with bacterial pyelonephritis, chronic osteomyelitis, and chronic bronchitis have also been shown to have them. Despite their rarity, thymine-dependent strains should be examined when clinical or microbiological recurrence is detected. There have been no cases of trimethoprim resistance caused by enzymatic changes in the drug , as it is the case with –lactamase inactivation of penicillin

From the Table (4-8) it was noted that Staphylococcus is resistant to penicillin F(100%) ,Trimethoprim (100%),and also to Enterobacteriaceae, and different bacteria It is highly susceptible to Ciprofloxacin, Nitrofurantoin, Levofloxacin .

In this study, highly resistant bacterium isolates obtained using a beta-lactam antibiotic (penicillin G) are related to three types of resistance mechanisms: (1) enzymatic degradation of antibacterial drugs, (2) alteration of bacterial proteins that are antimicrobial targets, and (3) changes in membrane permeability to antibiotics. Beta-lactamase synthesis degrades the amide bond of a beta-lactam ring. Enzymes (inhibit the ability of the antibiotic to attach to penicillin-binding proteins (PBPs) via limiting the antibiotic’s permeability through the outer cell membrane of bacteria) (decrease bactericidal action). The peptidoglycan layer is near to the organism’s surface in Gram-positive bacteria, therefore there are minimal obstacles to the medication reaching its target and changing PBPs. This explains why *S. aureus* is resistant to the majority of commercially available –lactams (Whalen *et al*., 2019).

The resistance to nitrofurantoin, levofloxacin and ciprofloxacin was 0%, which means that resistance was not obtained in this study.

Antibiotic usage (therapeutic, prophylactic , and growth promotion) in people and animals may have resulted in the selective rise of resistance in bacterial populations , as well as misuse and excessive use leads to increased resistance (Puah *et al*.,2013).

All samples were multi-drug resistant, as they were resistant to more than three classes of antibiotics, and some of them reached resistance to 11 antibiotics.

Antimicrobial resistance is a risk linked to high morbidity and mortality in bacterial pathogens (Pourbabaee *et al.,* 2017).

The results of this study indicate that meat sample 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 antibiotic-resistant pathogens that put people at risk. 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).

## 4.4.Molecular identify of *S. aureus* species specific *16SrRNA gene*

At this study, species specific *16SrRNA* was used for confirmation of identification of *S. aureus* isolates. After isolating 22 samples of *S. aureus* identified by species specific *16SrRNA*, the result was positive as shown in the Figure(4-9). The results show that 22 isolates of *S. aureus* gave positive results for16SrRNA as a single DNA band of PCR product with a molecular base of 791 bp.

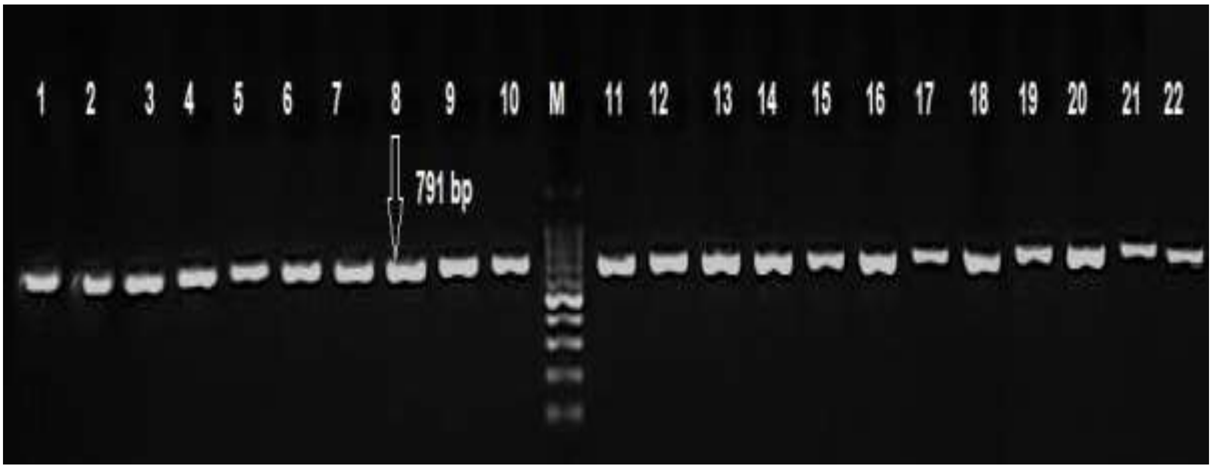

Figure (4-1):(1.5 %) Agarose gel electrophoresis at 70 volt for 60 minutes of PCR to gene species specific *16SrRNA* amplicon (791bp) ,( 1-22 )represented bacterial isolate , M ( DNA marker size (100bp).

The results presented were samples of fresh and canned meat, 22 (100%) which were positive for *S. aureus*.

## 4.5. Molecular detection of *S. aureus* enterotoxin genes

In this study, 5 primer coding for enterotoxin genes were tested , which were (Staphylococcal Enterotoxins A( *sea),* Staphylococcal Enterotoxins B (*seb),* Staphylococcal Enterotoxins C( *sec),* Staphylococcal Enterotoxins D ( *sed)* , Staphylococcal Enterotoxins E( *see*) Table (4-8).

Among the most dangerous types of bacteria isolated in this study is *S. aureus* and the high percentage of it. *Staphylococcus aureus* (*S. aureus*) is one of the major opportunistic foodborne pathogens as well as a source of human and animal infections. *S. aureus* is one of the common causes of food poisoning (Letertre *et al.*, 2003a). *S. aureus* is a common inhabitant of human nasal passages (Kluytmans *et al*., 1997) and as many as (30%) of the healthy human population carry the organism in their anterior nares (Nilsson and Ripa, 2006). The capacity of *S. aureus* to produce foodborne disease is regarded the world’s third most important cause of foodborne sickness. The ability of *S. aureus* to thrive and create

SEs under a variety of settings is demonstrated by the large range of foods involved in staphylococcal food poisoning (Morandi *et al.,* 2007). Enterotoxins from *S. aureus* is thermostable and resistant to gastrointestinal proteases such as pepsin, which explains their capacity to stay active after ingestion( Asao *et al*., 2003).

After the traditional diagnosis of the isolated samples, the specific samples belonging to the *S. aureus* was tested to ensure that they secrete Enterotoxins by using the traditional PCR technique.

PCR was done after the optimization step to select the specific PCR annealing temperature mention in Table(3-9) by gradient PCR thermocycler for 5 types of *S. aureus* toxin gene *sea* (127bp), *seb* (477) bp, *sec* (271) bp, *sed*(319) bp, and *see*(178 )bp. Of the 22 *S. aureus* isolates examined, 15 (68.18%) were found to be positive for more than one toxin genes.

the results revealed ,9(40.9%) strains possessed only one type of toxin gene (4 *sea*, 3 *seb*, 1 *sed*, , and 1 *sec*), while the remaining 6(27.27%) harbored more than one toxin gene1 (*sea* and *sed*),1 (*sea, seb, see),1(seb* and *sec),1(seb* and *sec), 1(seb, sec,* and *sec), 1(sea* and *seb ),1( sea* and *sec)* . The following ratios of enterotoxin genes were obtained by PCR there were 7 isolates which were harbored gene *sea* (31%), there which were 9 isolates were harbored gene *seb*(40.9%), there were 5 isolates which were harbored gene *sec* (22.7%) , there which were 2 isolates were harbored gene *sed* (9.09%), and there which were 2 isolates were harbored gene *see* (9.09%).The highest percentage of genes was *seb*.

The enterotoxins were reported as an important virulence agent involved in food poisoning (Asao *et al.*,2003). The production of enterotoxin from *S. aureus* is affected by different environmental factors such as temperatures, water activity, pH and other parameters.

Enterotoxins appears in 15 (68.18%) samples of *S.aureus* isolated from food samples. In another study, (Atanassova *et al*.,2001) Obtain similar proportions 57.1% and agree with (Vasconcelos *et al*.,2011) who found 54 (60·0%) of the 90 *S. aureus* isolates .

The result of the present study found that the percentage of ***sea*** gene was 7(31.8%) in *S. aureus* isolated from canned meat including: luncheon 2, sausage1, tuna 1, Minced meat 1, fresh meat 1, chicken pieces 1 Table (4-8). Figure (4-2).

Figure (4-2): (1.5 %) Agarose gel electrophoresis for( *sea* gene) Amplicon (127 bp) of *S. aureus* of PCR to( *sea* gene). M Lane represented 100 pb DNA ladder and the rest lane(4,5,8,10,16,17,21) represented *sea* gene positive (at 70 volt for 60 minutes).

The use of *sea* in food must be prevented due to its thermo-resistance propertyand gastrointestinal proteolytic enzymes such as pepsin and trypsin (Karimzadeh, & Ghassab, 2022). *sea* does not alter the flavor or color of food, therefore conventional cooking techniques do not get rid of this toxin. In most parts of the world, nearly 50% of foodborne diseases are caused by the *sea*.

This result agrees with (Yoon *et al*.,2022) who found that the percentage of *sea* was (32.6%) . This result is identical from the percentage of *sea* gene found by another study in the city of Karbala by (Muhammad and Alwan ,2017) who found that the percentage of ***sea*** were 12 (37.5%) of frozen, and 8 (32%) of meat samples fresh . and agrees with (Al-Ashmawy *et al*.,2016) who found that percentage of ***sea*** was 26.5%. in another study of (Zouharova and Rysanek ,2008) that detected *sea* in 27.1% of isolates s while the *seb* and *sec* genes were observed in 10% and 1.4% of the isolates. Similarly, 32.9% isolates of *S. aureus* positive for ***sea*** gene which were obtained from clinical specimen in Northern Iran (Mohseni *et al.,* 2018). In another study agree with (30.2% )isolates reveals staphylococcal enterotoxin A *(sea)* gene amplification (Aggarwal *et al*., 2019). Another study agrees with( Zhang *et al.,* 2022) who found ***sea*** gene (23.1%). Another study does not agree with our results( Hamdan *et al.,* 2022) who found the *sea* gene was observed among 120 (100%) clinical isolates.

The result of the present study that found the percentage of *seb* gene was 9 (40.9%), the highest percentage of enterotoxigenic genes was detected in this study including: luncheon 2, sausage1, tuna 2, chicken pieces 2, fresh meat 1, and hamburger 1 Table (4-7)(4-8), Figure (4-3).

Another researcher, (Shylaja *et al* ., 2018 )who found the prevalence of *seb* in *S. aureus* isolated from meat was 3(16.67) and( Savariraj *et al.,*2021) who found the ***seb*** gene the most prevalence . While (Asgarpoor *et al.*, 2018) who found the percentage of ***seb*** was (12.9%). While this does not agree with (Kurjogi *et al*.,2022) who not found the gene *seb* .

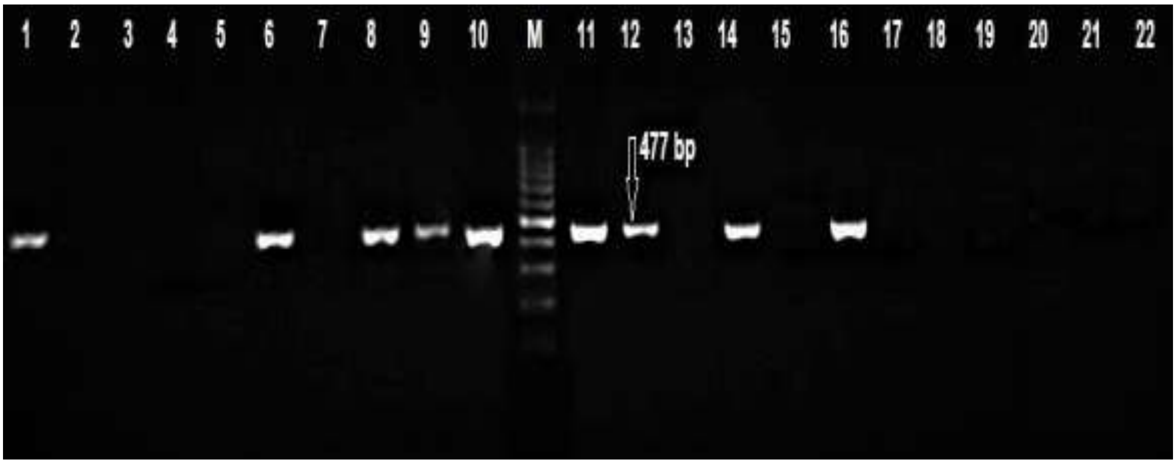

Figure (4-3): (1.5 %) Agarose gel electrophoresis for( *seb* gene) Amplicon (477bp) of *S. aureus* . M Lane represented 100 pb DNA ladder and the rest lane(1-6-8-9-10-11-12-14-16 ) represented *seb* gene positive (at 70 volt for 60 minutes).

The result of the present study found that the percentage of ***sec*** gene was 5 (22.7%), including : sausage1, Tuna 1, Minced meat 2, and hamburger1Table(4-7) (4-8), Figure (4-4).

This result agree with (Al-Ashmawy *et al.*,2016) who found that percentage of ***sec*** was 33.9%. Another researcher, (Roshan *et al* ., 2022 )who found that the prevalence of ***sec*** in *S. aureus* isolated from meat was (42.8%) and( Rall *et al* 2008) found the prevalence of ***sec*** in *S. aureus* 20.5% .

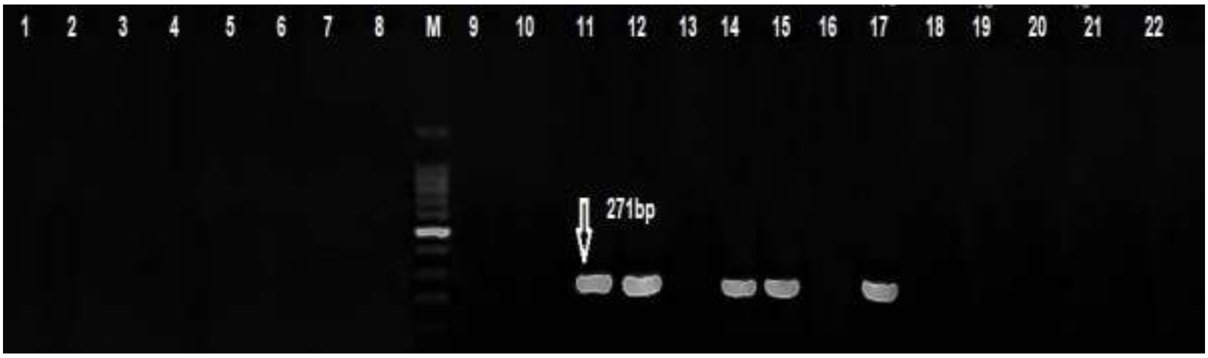

Figure (4-4): (1.5 %) Agarose gel electrophoresis for( *sec* gene) Amplicon (271bp) of *S. aureus* . M Lane represented 100 pb DNA ladder and the rest lane(11-12-14-15-17) represented *sec* gene positive (at 70 volt for 60 minutes).

The result of the present study found that the percentage of *sed* gene was 2(9.09%) in *S. aureus* isolated from canned meat including: Sausage 1, and tuna 1. Table (4-8)Figure (4-5).

This result agree with (Zhang *et al.*,2022) who found that the percentage of *sed* was (15.4%). The result does not agree with( Liao et al.,2022) who found that the percentage of *sed* was (1.4%). The result does not agree with (Omwenga, 2022) where none of the isolates tested positive for *sed.*

**Figure (4-5):** (1.5 %) Agarose gel electrophoresis for( *see* gene) Amplicon (319 bp) of *S. aureus* . M Lane represented 100 pb DNA ladder and the rest lane(4-18) represented *sec* gene positive (at 70 volt for 60 minutes).

The result of the present study found that the percentage of ***see*** gene was 2(9.09%) in *S. aureus* isolated from canned meat including: Tuna 2 Table (4-8)Figure (4-6).

This result does not agree with (Şanlıbaba,2022)where ***see*** genes were never detected. And also does not agree with (Omwenga *et al.*, 2019)who found that the most frequently encountered gene was ***see.***

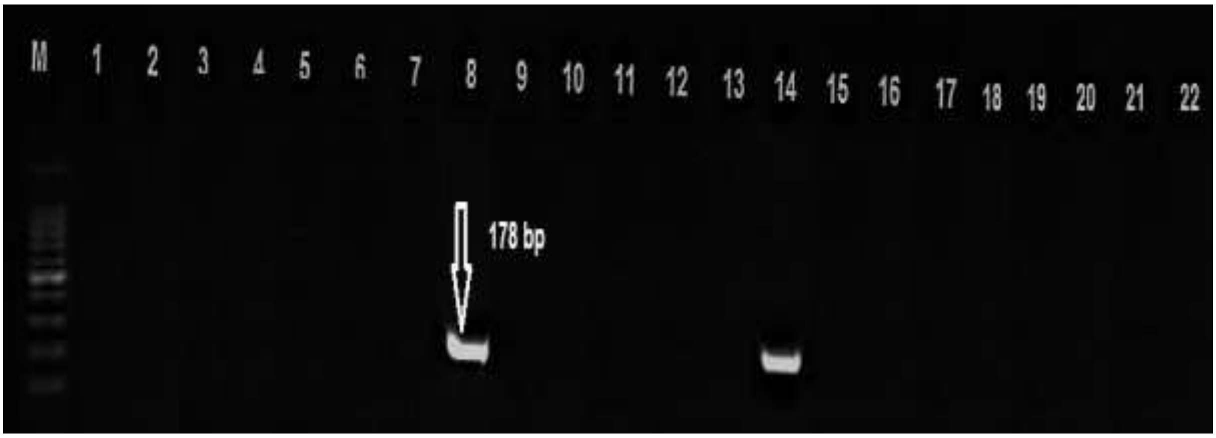

**Figure (4-6):** (1.5 %) Agarose gel electrophoresis for( *see* gene) Amplicon (178 bp) of *S. aureus* . M Lane represented 100 pb DNA ladder and the rest lane(8-14) represented *see* gene positive (at 70 volt for 60 minutes).

Other studies (Tarekgne *et al*., 2016 ; Mo Mohammed& El Dahshan,2016) also not detected of ***see*** gene , another study (Graciela *et al*.,2021) who Found the genes ***sea*** and ***see*** were not detected.

**Table (4-7 ): Types of negative and positive genes for all isolates of *Staphylococcus aureus***

| No | Type of sample | Gene name | | | | |
|---|---|---|---|---|---|---|
| | | ***Sea*** | ***Seb*** | ***Sec*** | ***Sed*** | ***See*** |
| **1** | **Luncheon** | - | + | - | - | - |
| **2** | **Minced meat** | - | - | - | - | - |
| **3** | **Grilled meat** | - | - | - | - | - |
| **4** | **Sausage** | + | - | - | + | - |
| **5** | **Luncheon** | + | - | - | - | - |
| **6** | **Chicken pieces** | - | + | - | - | - |
| **7** | **Grilled meat** | - | - | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| **8** | **Tuna** | + | + | - | - | + |
| **9** | **Luncheon** | - | + | - | - | - |
| **10** | **Fresh meat** | + | + | - | - | - |
| **11** | **Sausage** | - | + | + | - | - |
| **12** | **Hamburger** | - | + | + | - | - |
| **13** | **Luncheon** | - | - | - | - | - |
| **14** | **Tuna** | - | + | + | - | + |
| **15** | **Minced meat** | - | - | + | - | - |
| **16** | **Chicken pieces** | + | + | - | - | - |
| **17** | **Minced meat** | + | - | + | - | - |
| **18** | **Tuna** | - | - | - | + | - |
| **19** | **Fresh meat** | - | - | - | - | - |
| **20** | **Fresh meat** | - | - | - | - | - |
| **21** | **Luncheon** | + | - | - | - | - |
| **22** | **Fresh meat** | - | - | - | - | - |

**Table (4-8): Frequency of Enterotoxin encoding genes of *Staphylococcus aureus* isolated from meat, and meat product**

| | **Toxin gene** | | | | |
|---|---|---|---|---|---|
| | ***sea*** | ***seb*** | ***sec*** | ***sed*** | ***see*** |
| **luncheon** | **2(40%)** | **2(40%)** | **-----** | **-----** | **-----** |
| **sausage** | **1(50%)** | **1(50%)** | **1(50%)** | **1(50%)** | |
| **tuna** | **1(33.3%)** | **2 (66.6%)** | **1(33.3%)** | **1(33.3%)** | **2(66.6%)** |
| **chicken pieces** | **1(50%)** | **2(100%)** | **------** | **-------** | **------** |
| **Minced meat** | **1(33.3%)** | **-------** | **2(66.6%)** | **-------** | **-------** |
| **grilled meat** | **-------** | **-------** | **-------** | **------** | **------** |
| **Hamburger** | **------** | **1(100%)** | **1(100%)** | **------** | **------** |
| **fresh meat** | **1(25%)** | **1(25%)** | **-------** | **-------** | **------** |

# *Conclusions*

# *And*

# *Recommendations*

# Conclusions

- All canned samples were contaminated with different bacteria, but the most prevalent was *S. 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 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.

- The highest contamination of meat sample were Tazaj beef luncheon, Esalat chelavra, Chicken luncheon altazaj althabi, Sardines, Silou tuna and Chicken pieces nasma, With limits that exceeded allowed the permissible limits according FAO.

- The best medication for staphylococcal food poisoning were ciprofloxacin, Nitrofurantoin and Levofloxacin.

# Recommendations

1-Investigate the effectiveness of some plant extracts on foodborne *S. aureus.*

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

3- study *Staphylococcus lentus* and *Gemella sanguinis* because it appears for the first time in food.

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# *Appendix*

# *Appendix*

## Appendix (1):Types of canned goods and the names of their manufacturers

| No | Samples | Manufacturers | country | importing company | CFU |
|---|---|---|---|---|---|
| **1** | Chicken luncheon | Altaghziah | Produced by Al-taghziah S.A.L Beirut - Lebanon | Not found | $9.6\times10^8$ |
| **2** | Minced meat | local slaughter | Babylon | Not found | $8.5\times10^8$ |
| **3** | Luncheon Beef (Sham food) | Sham food company | Rural Damascus, Syria | Not found | $5.3\times10^8$ |
| **4** | minced meat | Tazaj / Amana Foods | The United Arab Emirates | qimat al_arz | $8.6\times10^8$ |
| **5** | Luncheon (Grand chicken ) | | Turkey | Not found | $4.7\times10^8$ |
| **6** | Sausage (Chicken franks ) | 7 tepesahne | Turkey | Not found | $3.8\times10^8$ |
| **7** | Sausage (Beef franks) | | Iran | Not found | Spread on the plate |
| **8** | Luncheon (Altunsa chicken ) | altunsa | Turkey | Not found | $2.8\times10^8$ |
| **9** | Luncheon( Hana chicken ) | Amana Foods | The United Arab Emirates | qimat al_arz | $5.6\times10^8$ |
| **10** | luncheon (Zwan chicken) | Zwanderful | Holland | Not found | $3.5\times10^8$ |
| **11** | luncheon (Tazaj beef) | Sham food | | qimat al_arz | $7.3\times10^8$ |
| **12** | minced meat (Esalat) | | Iran | Not found | $7.2\times10^8$ |
| **13** | Sausage( Esalat ) | | Iran | Not found | $9.4\times10^8$ |

# ***Appendix***

| | | | | | |
|---|---|---|---|---|---|
| **14** | Amrajona luncheon chicken | Taiba for food industry | turkey | Not found | $7.8 \times 10^{8}$ |
| **15** | Tuna baidar | | Thailand | Not found | $4.6\times10^{8}$ |
| **16** | Luncheon( Ayah ) | Taiba for food industry | Syria | Not found | $1.9\times10^{8}$ |
| **17** | Kasih tuna | Al- Kasih | Thailand | Not found | $3.7\ 10^{8}$ |
| **18** | luncheon (Al-fkhar chicken) | Gold food | Iraq | Not found | $1.3\times10^{8}$ |
| **19** | Luncheon( Jood chicken) | Jood chicken | United Arab Emirates | Balad al - asaleh | $5.4\times10^{8}$ |
| **20** | Hamburger | local slaughter | Babylon | Not found | $1.7\times10^{8}$ |
| **21** | Meat (Minced meat) | local slaughter | Babylon | Not found | $6.8\times10^{8}$ |
| **22** | Chicken (grilled meat) | local slaughter | Babylon | Not found | $8.4\times10^{8}$ |
| **23** | Altaghziah meet luncheon | Altaghziah | Beirut, Lebanon | Not found | $3.710^{8}$ |
| **24** | Luncheon (Heinz corned beef) | Heinz | Brazil | Al Farah International Company / Tunisia | $7.7\times10^{8}$ |
| **25** | Luncheon (Chicken altazaj althabi) | Rays food | Turkey | qimat al_arz | $4.08\times10^{9}$ |
| **26** | Minced meat Safa sardines | Dhofar Fisheries and Food Industries Company | Oman | Not found | $1.6\times10^{8}$ |
| **27** | Tuna Abu Al-Ward | | China | Al Farah International Company / Tunisia | $2.4\times10^{8}$ |
| **28** | (LuncheonBeef ) | Nabil Food | Jordan | Not found | $5.8\times10^{8}$ |

| | | | | | |
|---|---|---|---|---|---|
| | (martadella ) | Industries Company | | | |
| **29** | Siblou tuna | c- food international SAL | Thailand | Not found | $3.5 \times 10^8$ |
| **30** | Fulla tuna | c- food international SAL | Thailand | Al Farah International Company / Iraq | $3.4\times10^8$ |
| **31** | chicken pieces (Golden steak ) | Al Furat Company for Food Industries | Jordan | Halal Zad Company | $3.29\times10^9$ |
| **32** | chicken pieces (Crispy chicken nuggets) | Al Furat Company for Food Industries | Jordan | Halal Zad Company | $3.12\times10^9$ |
| **33** | chicken pieces (Chicken fillet jekor ) | Fostakia Food Industries Co. .Ltd | Iraq | Not found | $4.4 \times 10^8$ |
| **34** | chicken pieces( Smoked roast chicken ) | Nabil Food Industries Company | Jordan | Not found | $6.0\times10^8$ |
| **35** | chicken pieces (Chicken franks Americana ) | Kuwait food company Americana | Kuwait | Not found | $6.0 \times 10$ |
| **36** | Chicken pieces nasma | Nasma Almarai Food Industries .Co. Ltd | Iraq | Not found | $2.8\times10^8$ |
| **37** | chicken piemen sausage | Karbala Investment Authority | Iraq | Not found | $8.8\times10^8$ |
| **38** | chicken pieces( Chicken nuggets sadia ) | Al-Wafi Factory for Food Industries | The United Arab Emirates | Not found | $2.12\times10^9$ |
| **39** | Sausage sadia | Al-Wafi | The United Arab | Not found | $2.3\times10^8$ |

# *Appendix*

| | | | | | |
|---|---|---|---|---|---|
| | | Factory for Food Industries | Emirates | | |
| **40** | Meet piemen sausage | | | Not found | $2.0 \times 10^{8}$ |
| **41** | Alhaaela meet Hamburger | Alhaaela company | Iraq | Not found | $1.03\times10^{9}$ |
| **42** | grilled meat ( Alayle shawarma) | Alayle company | Iraq | Not found | Spread on the plate |
| **43** | Minced meat | Alhaaela company | Iraq | Not found | $5.2\times10^{8}$ |
| **44** | (chicken pieces) Chicken schnitzel | Banvit | Turkey | Not found | Spread on the plate |
| **45** | Minced meat Grinded meat | Alkafeel | Iraq | Not found | $1.3\times10^{8}$ |
| **46** | Hamburger (Chicken Burger) | Tip Top | | Not found | $6.2\times10^{8}$ |
| **47** | Hamburger (Meat Burger ) | Al- Haaela | Iraq | Not found | $4.8\times10^{8}$ |
| **48** | chicken pieces( Chicken nuggets )(mini chef ) | Sadia (al-wafi food ) | United Arab Emirates | Not found | $1.31\times10^{9}$ |
| **49** | Breaded chicken golden bites | BRF S.A. | Brazil | Not found | $7.3\times10^{8}$ |
| **50** | chicken pieces( Chicken nuggets ) | Alhaealh | Iraq | Not found | Spread on the plate |
| **51** | (grilled meat )Chicken gold steak | Alhaealh | Iraq | Not found | $4.8\times10^{8}$ |
| **52** | Goddess tuna | OEM | Thailand canned | Not found | $4.7\times10^{8}$ |

| | | | | | |
|---|---|---|---|---|---|
| | | | tuna | | |
| **53** | grilled meat (Chicken nuggets ) | Americana | Kuwait | Not found | $3.9\times10^{8}$ |
| **54** | Sausage | Americana | Kuwait | Not found | $6.8\times10^{8}$ |
| **55** | Sausage | Banvit | Turkey | Not found | $5.8\times10^{8}$ |
| **56** | grilled meat (Golden steak ) | Durra Karbala | Jordan | Not found | $2.1\times10^{8}$ |
| **57** | Hamburger (Beef burger ) | Pemina | Iraq | Not found | $4.5\times10^{8}$ |
| **58** | Sea bell tuna | OEM | Zhejiang, China | Not found | $3.3\times10^{8}$ |
| **59** | Hamburger (Beef burger) | Almaraei food | Iraq | Not found | Spread on the plate |
| **60** | Blue lagoon tuna | OEM | Zhejiang, China | Not found | $2.1\times10^{8}$ |

## Appendix (2): Type of bacteria according to diagnosis Vitek 2 compact result

Selected Organism: *Burkholderia cepacia* group

| | | |
|---|---|---|
| Identification information | Analysis time 4.90 hors | Status final |
| Selected Organism | 90 % probability *Burkholderia cepacia* group<br>Bionumber : 2341614450000000 | |
| ID Analysis Messages | | |

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | + | 4 | PyrA | - | 5 | lARL | + | 7 | dCEL | + | 9 | BGAL | - |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | + | 13 | dGLU | + | 14 | GGT | - | 15 | OFF | - |
| 17 | BGLU | - | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | - | 22 | BAlap | - |
| 23 | ProA | - | 26 | LIP | - | 27 | PLE | +- | 29 | TyrA | - | 31 | URE | - | 32 | dSOR | + |
| 33 | SAC | + | 34 | dTAG | - | 35 | dTRE | + | 36 | CIT | - | 37 | MNT | - | 39 | 5KG | - |
| 40 | ILATK | - | 41 | AGLU | - | 42 | SUCT | - | 43 | NAGA | - | 44 | AGAL | - | 45 | PHOS | - |
| 40 | GLyA | - | 47 | ODC | - | 48 | LDC | - | 53 | IHISa | - | 56 | CMT | - | 57 | BGUR | - |
| 58 | O129R | - | 59 | GGAA | - | 61 | IMLTA | - | 62 | ELLM | - | 64 | ILATa | - | | | |

Selected Organism: *Staphylococcus lentus* group

| | | |
|---|---|---|
| Identification information | Analysis time 4.90 hors | Status final |
| Selected Organism | 87 % probability *Staphylococcus lentus* group<br>Bionumber : 512012421761331 | |
| ID Analysis Messages | | |

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | AMY | + | 4 | PIPLC | - | 5 | dXYL | + | 8 | ADHI | + | 9 | dCEL | + | 9 | BGAL | - |
| 13 | APPA | - | 14 | CDEX | + | 15 | AspA | - | 16 | BGAR | - | 17 | GGT | - | 15 | OFF | - |
| 20 | LeuA | - | 23 | ProA | - | 24 | BGURr | - | 25 | AGAL | - | 26 | BXYL | - | 22 | BAlap | - |
| 28 | AlaA | - | 29 | TyrA | - | 30 | dSOR | + | 31 | URE | - | 32 | URE | - | 32 | dSOR | + |
| 38 | dRIB | + | 39 | ILATK | - | 42 | LAC | - | 44 | NAG | + | 45 | MNT | - | 39 | 5KG | - |
| 47 | NOVO | - | 50 | NC6.5 | + | 52 | dMAN | + | 53 | dMNE | + | 54 | AGAL | - | 45 | PHOS | - |
| 57 | dRAF | + | 58 | O129R | + | 59 | SAL | - | 60 | SAC | + | 62 | CMT | - | 57 | BGUR | - |
| 64 | OPTO | + | | | | | | | | | | | | | | | |

# ***Appendix***

Selected Organism: *Enterobacter cloacae* group

| | | |
|---|---|---|
| Identification information | Analysis time 4.90 hors | Status final |
| Selected Organism | 87 % probability ***Enterobacter cloacae*** group<br>Bionumber : 0627735353513210 | |
| ID Analysis Messages | | |

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | lARL | - | 7 | dCEL | + | 9 | BGAL | + |
| 10 | H2S | - | 11 | BNAG | + | 12 | AGLTp | - | 13 | dGLU | + | 14 | GGT | + | 15 | OFF | + |
| 17 | BGLU | + | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | + | 22 | BAlap | - |
| 23 | ProA | + | 26 | LIP | - | 27 | PLE | +- | 29 | TyrA | + | 31 | URE | + | 32 | dSOR | - |
| 33 | SAC | + | 34 | dTAG | - | 35 | dTRE | + | 36 | CIT | + | 37 | MNT | + | 39 | 5KG | - |
| 40 | ILATK | + | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | + | 44 | AGAL | - | 45 | PHOS | - |
| 46 | GLyA | + | 47 | ODC | + | 48 | LDC | - | 53 | IHISa | - | 56 | CMT | + | 57 | BGUR | - |
| 58 | O129R | + | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

Selected Organism: *Streptcoccus agalactiae* group

| | | |
|---|---|---|
| Identification information | Analysis time 4.90 hors | Status final |
| Selected Organism | 90 % probability *Streptcoccus agalactiae* group<br>Bionumber : 071412367773631 | |
| ID Analysis Messages | | |

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | AMY | - | 4 | PIPC | - | 5 | dXYL | - | 8 | ADHI | + | 9 | BGAL | + | 9 | AGLU | + |
| 13 | APPA | + | 11 | CDEX | - | 15 | AspA | - | 16 | BGAR | - | 17 | AMAN | - | 15 | PHOS | + |
| 20 | LeuA | + | 18 | ProA | - | 24 | BGURr | - | 25 | AGAL | - | 26 | PYrA | + | 22 | BGUR | - |
| 28 | AlaA | + | 26 | TyrA | + | 30 | dSOR | - | 31 | URE | - | 32 | POLB | + | 32 | dGAL | + |
| 38 | dRIB | + | 34 | ILATK | + | 42 | LAC | + | 44 | NAG | + | 45 | dMAL | + | 39 | BACL | + |
| 47 | NOVO | + | 50 | NC6.5 | + | 52 | dMAN | + | 53 | dMNE | + | 54 | MBdG | + | 45 | PUL | - |
| 57 | dRAF | - | 58 | O129R | + | 59 | SAL | + | 60 | SAC | + | 62 | dTRE | + | 57 | ADH2s | - |
| 64 | OPTO | + | | | | | | | | | | | | | | | |

# *Appendix*

Selected Organism: *Micrococcus luteus* group

| | | |
|---|---|---|
| Identification information | Analysis time 7.83 hours | Status final |
| Selected Organism | 95 % probability<br>Bionumber : | *Micrococcus luteus* group<br>010032112000000 |
| ID Analysis Messages | | |

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | AMY | - | 4 | PIPC | - | 5 | dXYL | - | 8 | ADHI | + | 9 | BGAL | - | 11 | AGLU | - |
| 13 | APPA | - | 11 | CDEX | - | 15 | AspA | - | 16 | BGAR | - | 17 | AMAN | - | 19 | PHOS | - |
| 20 | LeuA | + | 18 | ProA | + | 24 | BGURr | - | 25 | AGAL | - | 26 | PYrA | + | 27 | BGUR | - |
| 28 | AIaA | + | 26 | TyrA | - | 30 | dSOR | - | 31 | URE | + | 32 | POLB | - | 37 | dGAL | - |
| 38 | dRIB | - | 34 | ILATK | + | 42 | LAC | - | 44 | NAG | - | 45 | dMAL | - | 46 | BACL | - |
| 47 | NOVO | - | 50 | NC6.5 | - | 52 | dMAN | - | 53 | dMNE | - | 54 | MBdG | - | 56 | PUL | - |
| 57 | dRAF | - | 58 | O129R | - | 59 | SAL | - | 60 | SAC | - | 62 | dTRE | - | 63 | ADH2s | - |
| 64 | OPTO | - | | | | | | | | | | | | | | | |

Selected Organism: *Klebsiella pneumonia* group

| | | |
|---|---|---|
| Identification information | Analysis time 4.00 hors | Status final |
| Selected Organism | 99 % probability<br>Bionumber : | *Klebsiella pneumonia* group<br>2207734653164010 |
| ID Analysis Messages | | |

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | + | 4 | PyrA | - | 5 | lARL | - | 7 | dCEL | + | 9 | BGAL | - |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | + | 14 | GGT | + | 15 | OFF | + |
| 17 | BGLU | + | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | + | 22 | BAlap | - |
| 23 | ProA | - | 26 | LIP | - | 27 | PLE | +- | 29 | TyrA | - | 31 | URE | + | 32 | dSOR | + |
| 33 | SAC | + | 34 | dTAG | - | 35 | dTRE | + | 36 | CIT | + | 37 | MNT | + | 39 | 5KG | - |
| 40 | ILATK | + | 41 | AGLU | - | 42 | SUCT | - | 43 | NAGA | - | 44 | AGAL | + | 45 | PHOS | + |
| 46 | GLyA | - | 47 | ODC | - | 48 | LDC | + | 53 | IHISa | - | 56 | CMT | - | 57 | BGUR | - |
| 58 | O129R | + | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

# Appendix

Selected Organism: *Staphylococcus aureus* group

| Identification information | Analysis time 4.90 hors | Status final |
|---|---|---|
| Selected Organism | 98 % probability *Staphylococcus aureus* group<br>Bionumber : 320854221568451 | |
| ID Analysis Messages | | |

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | AMY | - | 4 | PIPC | - | 5 | dXYL | - | 8 | ADH1 | + | 9 | BGAL | - | 11 | AGLU | + |
| 13 | APPA | - | 11 | CDEX | - | 15 | AspA | - | 16 | BGAR | - | 17 | AMAN | - | 19 | PHOS | - |
| 20 | LeuA | - | 23 | ProA | - | 24 | BGURr | - | 25 | AGAL | - | 26 | PYrA | + | 27 | BGUR | - |
| 28 | AlaA | - | 29 | TyrA | - | 30 | dSOR | - | 31 | URE | - | 32 | POLYB | + | 37 | dGAL | - |
| 38 | dRIB | - | 39 | ILATK | + | 42 | LAC | - | 44 | NAG | + | 45 | dMAL | + | 46 | BACL | + |
| 47 | NOVO | - | 50 | NC6.5 | + | 52 | dMAN | + | 53 | dMNE | + | 54 | MBdG | + | 56 | PUL | - |
| 57 | dRAF | - | 58 | O129R | + | 59 | SAL | - | 60 | SAC | + | 62 | dTRE | + | 63 | ADH2s | - |
| 64 | OPTO | + | | | | | | | | | | | | | | | |

Selected Organism: *kocuria kristinae* group

| Identification information | Analysis time 5.87 hours | Status final |
|---|---|---|
| Selected Organism | 93 % probability *kocuria kristinae* group<br>Bionumber : 014032302000030 | |
| ID Analysis Messages | | |

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | AMY | - | 4 | PIPC | - | 5 | dXYL | - | 8 | ADH1 | + | 9 | BGAL | - | 11 | AGLU | - |
| 13 | APPA | - | 11 | CDEX | - | 15 | AspA | + | 16 | BGAR | - | 17 | AMAN | - | 19 | PHOS | - |
| 20 | LeuA | + | 23 | ProA | + | 24 | BGURr | - | 25 | AGAL | - | 26 | PYrA | + | 27 | BGUR | - |
| 28 | AlaA | + | 29 | TyrA | + | 30 | dSOR | - | 31 | URE | - | 32 | POLYB | - | 37 | dGAL | - |
| 38 | dRIB | - | 39 | ILATK | + | 42 | LAC | - | 44 | NAG | - | 45 | dMAL | - | 46 | BACL | - |
| 47 | NOVO | - | 50 | NC6.5 | - | 52 | dMAN | - | 53 | dMNE | - | 54 | MBdG | - | 56 | PUL | - |
| 57 | dRAF | - | 58 | O129R | - | 59 | SAL | - | 60 | SAC | + | 62 | dTRE | + | 63 | ADH2s | - |
| 64 | OPTO | - | | | | | | | | | | | | | | | |

# Appendix

Selected Organism: *Gemella sanguinis* group

| | | |
|---|---|---|
| Identification information | Analysis time 5.57 hours | Status final |
| Selected Organism | 98 % probability *Gemella sanguinis* group<br>Bionumber : 004402500140030 | |
| ID Analysis Messages | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biochemical Details | | | | | | | | | | | | | | | | | |
| 2 | AMY | - | 4 | PIPC | - | 5 | dXYL | - | 8 | ADH1 | - | 9 | BGAL | - | 11 | AGLU | - |
| 13 | APPA | - | 14 | CDEX | - | 15 | AspA | + | 16 | BGAR | - | 17 | AMAN | - | 19 | PHOS | + |
| 20 | LeuA | - | 23 | ProA | - | 24 | BGURr | - | 25 | AGAL | - | 26 | PYrA | + | 27 | BGUR | - |
| 28 | AlaA | + | 29 | TyrA | - | 30 | dSOR | + | 31 | URE | - | 32 | POLYB | - | 37 | dGAL | - |
| 38 | dRIB | - | 39 | ILATK | - | 42 | LAC | - | 44 | NAG | + | 45 | dMAL | - | 46 | BACL | - |
| 47 | NOVO | - | 50 | NC6.5 | - | 52 | dMAN | + | 53 | dMNE | - | 54 | MBdG | - | 56 | PUL | - |
| 57 | dRAF | - | 58 | O129R | - | 59 | SAL | - | 60 | SAC | + | 62 | dTRE | + | 63 | ADH2s | - |
| 64 | OPTO | - | | | | | | | | | | | | | | | |

## الخلاصة :

سلامة الغذاء هو عنصر حيوي لضمان مستوى معيشي مرتفع. الغذاء هو أحد أهم الاحتياجات الأساسية لبقاء الإنسان. يوفر للأفراد الطاقة والتغذية التي يحتاجون إليها ليكونوا أصحاء ومنتجين. ومع ذلك ، فإن الطعام الذي لم يتم فحصه بدقة قد يحتوي على بكتيريا ضارة. قد يتسبب التلوث بالكائنات الدقيقة أو المواد الكيميائية في حدوث مشكلات صحية للإنسان. هذه العدوى المنقولة بالغذاء ، والتي تُعرف أحيانًا بالتسمم الغذائي ، هي نوع من الأمراض التي تحدث عند تناول الطعام. تخدم سلامة الأغذية عددًا من الأغراض ، مثل حماية البشر من العدوى المنقولة عن طريق الأغذية.

الهدف من هذه الدراسة هو عزل البكتيريا المختلفة الموجودة في اللحوم ومنتجات اللحوم (المعلبة) ، وعزل المكورات العنقودية الذهبية والتحقق من قابليتها للمضادات الحيوية وانتشار الجين المعوي ( *sea, seb, sec, sed* and, *see*) فيها.

تم زرع جميع العينات على أجار المغذيات لإجمالي عدد البكتيريا. تم زراعة العزلات بعد ذلك على MacConkey ، mannitol salt agar، blood agar، eosin methylene blue، ثم تم تقديمها لنظام Vitek 2 المضغوط للتاكد منها ودراسة المضادات وفقًا لـ CLSI 2021. تم فحص جينات Staphylococcus Enterotoxin بواسطة تفاعل البلمرة المتسلسل.

إجمالي 87 عينة عشوائية ، ستون عينة لكل من لانشون 15 (17.24٪) ، لحن 9 (10.34٪) ، قطع دجاج 9 (10.34٪) ، لحم مفروم 7 (8.04٪) ، لحم مشوي 5 (5.74٪) ، همبرغر 5 (5.74٪) ، سجق 10 (11.49٪) ، لحوم طازجة 27 (31.03٪) من بعض محلات السوبر ماركت في مدينة الحلة في العراق ، كانت (80) عينة نمو على أجار المغذيات و 7 (8.04٪) عينة غير نمو من طازجة لحم

أظهرت النتائج المتحصل عليها أن اختبار الحساسية لمضادات الميكروبات لـ 22 عزلة من بكتريا *S. aureus* ضد المضادات الحيوية المختلفة أظهر بوضوح حساسية عالية للسيبروفلوكساسين (CIP) 22 (100٪) ، ليفوفلوكساسين (LEV) 22 (100٪) ، نتروفورانتوين (F300) 22 (100)، الجنتاميسين (90.9٪) يليه ريفامبيسين (86.36٪) ، بينما لوحظ مقاومة عالية ضد البنسلين 22 (100٪) ، Trimethoprim 22 (100٪) ، يليه الإريثروميسين (86.6٪)، الكليندامايسين (AD) (86.6٪) ، الكلورامفينيكول (C) (77.27٪) ، التتراسيكلين (68.81٪)، أوكساسيللين (63.63٪)

علاوة على ذلك ، تم الكشف عن الجين ES في عزلات *S. aureus* عن طريق الفحص الجزيئي وظهر المجموع (n = 22) من *S. aureus* the *sea* gene 7 (31٪)، *seb* 9 (40.9٪)، *sec* 5 (22.7٪)، *sed* 2 (9.09٪)، انظر 2 (9.09٪). إن وجود السم المعوي في سلالات المكورات العنقودية الذهبية ، خاصة بين

منتجات اللحوم المصنعة ، ينذر بالحاجة إلى التطبيق الصارم للتدابير الصحية المناسبة وممارسات التصنيع الجيدة (GMPs) أثناء تحضير الطعام ومناولته وتخزينه.

تضمنت الدراسة الحالية عزل البكتيريا المسببة للأمراض الأكثر شيوعا في اللحوم واللحوم المعلبة وهي *Staphylococcus aureus* ودراسة جيناتها المعوية ومقاومتها للمضادات الحيوية.

جمهورية العراق

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# توصيف بكتريا المكورات العنقودية المعزولة من عينات اللحوم في مدينة الحلة

**رسالة مقدمة الى**

**مجلس كلية العلوم / جامعة بابل وهي جزء من متطلبات نيل شهادة الماجستير في العلوم / علوم الحياة**

**من قبل**
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