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Study the Bacterial Contamination of Frozen Food in Local Markets of Misan Governorate /South of Iraq

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By

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بسم الله الرحمن الرحيم

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Dedication

To the shrine of the owner of the age and time,

the awaited Imam Mahdi

To the reason of what I become today ,the candle of my life.

my parents

may God have mercy on them

To the one who loved, encouraged and supported me,

my dear husband

To the reason for my joy in life,

my brother, sister and my children

Duaa

Abstract

The present study was undertaken to isolate and characterize some types of bacteria from frozen foods by biochemical and molecular methods. During a six months period between November 2020 to April 2021, 100 samples of frozen food were collected randomly from the commercial markets in Misan Governorate and then transferred under sterile conditions to microbiology laboratory at science college. The frozen food samples included: Meat products from local and imported companies ,represented by (burger – sausage – kebab – shawarma – minced meat) and Chicken products from local and imported companies ,represented by (chest – thigh- liver- burger- kebab) where take ten samples for each product used .

The results show that the imported burger ,sausage and shawarma samples had mean value $2.52\times10^{13}{}^{b}\pm1.44\times10^{13}$, $3.86\times10^{13}{}^{b}\pm2.92\times10^{13}$and $2.78\times10^{13}{}^{a}\pm1.12\times10^{13}$respectively while the local samples have mean value which were $2.32\times10^{13}{}^{b}\pm1.06\times10^{13}$, $2.85\times10^{13}{}^{b}\pm0.16\times10^{13}$ and $2.99\times10^{13}{}^{a}\pm2.18\times10^{13}$ respectively ,so the imported samples of these three types of meat products had highest value of Aerobic Plate Count than local samples. The minced meat samples from local company(L1) have mean value $5.56\times10^{12}{}^{a}\pm3.10\times10^{12}$more than the samples of local company (L2) which have $2.59\times10^{13}{}^{a}\pm0.4\times10^{13}$, kebab from local company (L1) has mean value $2.46\times10^{13}{}^{b}\pm1.81\times10^{13}$while the samples from local company (L2) which have$1.24\times10^{13}{}^{b}\pm0.19\times10^{13}$ mean value so the kebab samples from L1 have highly aerobic plate count than the samples from L2.

The results of frozen poultry meat cuts and products showed that the imported liver ,chest ,thighs and burger samples had mean value which $1.89\times10^{13}{}^{b}\pm1.22\times10^{13}$, $2.91\times10^{13}{}^{b}\pm2.16\times10^{13}$, $2.78\times10^{13}{}^{b}\pm1.26\times10^{13}$ and $2.59\times10^{13}{}^{b}\pm2.07\times10^{13}$ respectively while the local samples of these type have mean $9.2\times10^{12}{}^{b}\pm4.7\times10^{12}$, $1.83\times10^{13}{}^{b}\pm1.19\times10^{13}$, $1.64\times10^{13}{}^{b}\pm0.13\times10^{13}$ and $1.66\times10^{13}{}^{b}\pm1.29\times10^{13}$

respectively so the imported samples had highest values of aerobic plate count. The chicken kebab samples from local company (L1) have mean value 2.09×10^{13} ± 1.23×10^{13} while the samples from local company (L2) have 2.23×10^{13} ± 1.49×10^{13}mean value so the aerobic plate count of kebab samples from L2 is more than the aerobic plate count of L1 samples.

Many bacterial strains were isolated from frozen food (meat and chicken products). Forty one of bacterial species isolates were characterized at biochemical levels by ordinary and standard bacteriological tests, preliminary test showed that Gram negative bacteria was most common in meat products (52%) while Gram positive bacteria represents 48% . In poultry samples Gram stain showed that Gram positive bacteria was most dominant (59%) with (41%) the percentage of Gram negative bacteria. The isolates bacteria were further identified by the Vitek-2 system and the molecular analysis. Only five bacterial species misidentified by Vitek2compact system and identified by used monoplex PCR.

Multiplex PCR and monoplex PCR were used for further identification of bacteria. Multiplex PCR was used to diagnose the presence of common types of bacteria in food samples by special primers, and it gave negative results which was identical to the preliminary tests as well as the Vitek2 system , and confirmed that the samples did not contain these types , while monoplex PCR was used to diagnose eighteen types of bacteria strain isolated using a universal primer (16S r RNA) that gave 1500 bp amplification product. Nucleotide sequences were studied at the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (Nucleotide BLAST).

The diagnosed bacteria were *Aeromonas veronii, Pseudomonas plecoglossicida, Acinetobacter lwofii, Aeromonas veronii, Klebsiella pneumoniae, Pseudomonas japonica, Pseudomonas songnenensis, Klebsiella pneumoniae* subsp.ozaenae, *Psychrobacter sagnuinis, Klebsiella pneumoniae, Acinetobacter lwofii,*

Lysinibacillus boronitolerans, Bacillus licheniformis, Enterobacter hormaechei, Pseudomonas putida, Serratia liquefaciens, Comamonas testosterone ,and *Methylogaea oryzae*.

Sixteen bacterial isolates were recorded in Gene Bank under different accession numbers: MZ934693.1, MZ934671.1, MZ931306.1, MZ931286.1, MZ930472.1, MZ927456.1, MZ927229.1, MZ923509.1, MZ923503.1, MZ921931.1, MZ920155.1,MZ919358.1,MZ919316.1,MZ919316.1,MZ913024.1,MZ911849.1.

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List of Abbreviations

Abbreviations	Key
+ve	Gram positive
APC	Aerobic Plate Count
AST	Antibiotic Susceptibility Test
bp	base pair
C˚	Celsius degree
CDC	Centers for Disease Control and prevention
CFU	Colony Form Unit
Csp	Cold shock proteins
ctx	*Cholera* toxin gene
DNA	Deoxyribonucleic acid
EDTA	Ethylendiemintetraacetate
EMB	Eosin Methyl Blue
FDA	Food and Drug Adminstration
hly	Hemolysin gene of *Listeria monocytogenes*
I	Import
invA	Invasion gene of *Salmonella*
L	Local

M	Meat
Max	Maximum
min	minute
Min	Minimum
ml	Milliliter
mm	millimeter
mPCR	multiplexPolymerase Chain Reaction
n	number
NCBI-BLAST	National Center for Biotechnology Information-Basic Local Alignment Search Tool
nm	nanometer
nuc	Thermostable nuclease gene *Staphylococcus aureuse*
PCR	Polymerases Chain Reaction
PH	Power of Hydrogen
pmole	Pecomole
p	probare
P	Poultry
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
rpm	rotation per minute
rRNA	ribosomal Ribonucleic acid
sec	second
SPSS	Social Package of Social Sciences
stx	Gene of *E .coli* O157:H7

Std Dev	Standard Deviation
TBE	Tris Borate EDTA
TCBS	Thiosulfate Citrate Bile Sucrose
tlh	Thermolabile hemolysin gene of *V.parahaemolyticus*
TNTC	Too Numerous To Count
tRNA	transfer Ribonucleic acid
U.S	United States
UV	Ultra Violet
-ve	Gram negative
Vitek	Vitality index of traditional environmental knowledge
WHO	The World Health Organization
µg/gm	Microgram/gram
µl	Microliter
MALDI TOF MS	Matrix Assisted Laser Desorption /Ionization Time of Flight Mass spectrometry

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CHAPTER ONE

INTRODUCTION

Introduction:-

Meat is one of the most important food sources for humans , which is characterized by its high nutritional value and pleasant taste and it's considered as one of the most desirable food by the consumer (Hui *et al.*, 2001and Hafez *et al.*, 2019). Meat is deem one of the most perishable foods and it is a suitable environment for the growth and reproduction of spoilage and pathogenic bacteria due to its high nutritional concentrations , suitable moisture and high water activity (Húngaro *et al.*, 2016).

Over 250 known food-borne diseases could be caused by food contaminated with parasites, viruses, bacteria, and toxins, which are continuing to be a public health problem in the world , of these, bacteria cause a large portion (approximately 90%) of all food-borne illnesses(Ahmed *et al.*, 2014).

Food contamination by foodborne pathogens is a serious public health concern one that could lead to foodborne diseases (Akeda, 2015). Foodborne diseases are continuously being a global public health problem with an estimation of 600 million people getting ill yearly (Srey *et al.*, 2013 and WHO, 2020). Yang *et al.* (2017) indicated that food contamination may happen during any stage in the farm-to-fork continuum from animal, human, or environmental sources and cause foodborne disease and food poisoning .

The two primary types of bacteria that consequence are spoilage bacteria and pathogenic bacteria. Spoilage bacteria are normally not harmful, but they are cause food to lose quality or deteriorate by developing a bad smell or texture like ***Pseudomonas* spp., *Enterobacteriaceae* , *Brochothrix thermosphacta* ,*Lactic acid*** bacteria according to Pennacchia *et al.*, (2011) while Pathogenic bacteria are those such as ***Salmonella, Escherichia coli* O157:H7, *Campylobacter jejuni, Staphylococcus aureus* , *and Listeria monocytogenes,*** all which cause food-borne illness and can't be seen or smelled (Abbas and Alghanim, 2016).

Meat can be stored safely for extended periods of time by applying the correct hygienic procedures and some of these procedures include the places, surfaces and tools which are used in the meat processing should have a high level of cleanliness and also its workers (Chellaiah *et al*., 2019). In order to preserve food, microbial enzymes and the natural enzymes found in food must be prevented from damaging it (Ledward, 2003 and Rajendran *et al*., 2019).

The most commonly used tools of standard methods for pathogen detection are immunological based method, cultural based method, and molecular based methods. The fast and precise identification of bacterial pathogens is important, both for quality assurance and to trace bacterial pathogens within food supplies (Yang *et al*., 2013).

In recent years, Iraqi local markets were taken over by a variety of frozen meat and poultry product from different well-known and unknown origins regardless of whether such food is valid for human consumption or not. Also, the lack of proper requirements and equipment of transportation, storing and marketing such food may result in the contamination of food with various biological, chemical, and physical contaminants that may form serious health threats specifically in those imported and local products.

This study was aimed to:-

Determine the percentage of the frozen food contamination with bacteria available in the public markets of Misan Governorate/Iraq and this was achieved through:

a- Isolation of various species of bacteria of some frozen food including local and imported retail meat and chicken.

b- Phenotypic study of isolated bacteria by using conventional and confirmed laboratory techniques.

c- Molecular study of isolated bacteria by using specific and 16s rRNA primers using multiplex PCR and monoplex PCR respectively.

CHAPTER TWO

LITERATURE REVIEW

2. Literature Review:-

2.1. Food Contamination :-

Food contamination happens when bacteria or other germs enter food and make them unsafe to human consumptions. Also it can refer to meals that have rotted due to the presence of microorganisms that render them unsafe for consumers . Food safety is compromised when foodstuffs get contaminated with a potentially hazardous agent (Sadiku *et al*., 2020). As explained by Sadiku *et al.,(*2020), food contamination can be divided into three types :-

1-Biological Contamination: Occurs when fungal, bacteria, or other damaging microorganisms contaminate food. It is a common cause of food-borne illness, food spoilage, and food poisoning. Although all foods can bear dangerous pathogens, some foods are rather more vulnerable to biological contamination than others ones.

2-Chemical Contamination: Refers to food that has been contaminated by a type of chemical substance. Common sources of chemical contamination include unwashed fruits, kitchen equipment, agrochemicals ,vegetables, and food containers made of non-safe plastics. Also, Nuclear accidents can lead to widespread environmental contamination and can cause death.

3-Physical Contamination: Takes place once physical objects enter food. Common sources of physical contamination include fingernails, glass, hair, metals, pests, jewelry, and dirt.

The World Health Organization (WHO) recognizes food contamination as a global challenge in various documents and reports (WHO, 2015 and Fukuda *et al*., 2015). It is clearly acknowledged in a statement "food contamination that occurs in one place may affect the health of consumers living on the other side of the planet" (Alert, 2014).

Hussain (2016) displayed that food contamination was the major challenges of the

future, there are vast majorities of people whom experience a waterborne or food borne disease at some point in their lives worldwide and therefore consumption of contaminated foods may cause illness in many people and many of them die as a result of it. This scenario makes "food contamination" a serious problem. The list of food contamination challenges is extremely long and it keeps growing. Many types of food could be contaminated like meat, meat products, poultry, dairy, fruits and vegetables (Fukuda *et al*., 2015).

2.2. Bacterial Contamination of Meat :-

Meat and meat products in general have a major role in human nutrition as they are desirable foodstuffs. They are an important source of protein, minerals, essential amino acids, fat, vitamins and other nutrients essential for human beings (Biesalski, 2005). On the other hand, they are considered to be an ideal culture medium for the growth of many organisms owing to their high content of moisture, abundance of minerals, nitrogenous compounds of various degree of complexity, accessory growth factors and some fermentable carbohydrates of a favorable pH as glycogen (Mohammed, 2011 and Eid *et al.,* 2018*)*.

In most cases, the internal meat tissues of a healthy animal free of diseases do not contain any of the microorganisms. The animal's skin, as well as the lining membranes of the internal viscera, form protective covers against the invasion of microorganisms to the internal tissues, in addition to the defenses found in the animal's body fluids, tissues and glands, such as white blood cells and antibodies . But contamination of these internal tissues may occur in cases of the animal's exposure to physical stress processes (extreme thirst or hunger, or sudden heat changes) that affect the animal's immune system and lead to weak body resistance and a defect in the permeability of some membranes, allowing the passage of microorganisms to the muscles through the blood vessels located in that area, this is what is called the primary contamination of meat (Lund *et al*., 2000).

The secondary contamination of meat with microorganisms may be happen by meat handlers, which they may have carried the pathogenic microorganism through the

processes of manufacturing, marketing and packing. Inappropriate cooking, poor hygiene through the production processes, refrigeration or the retail and storage of foods may cause meat borne illnesses and food poisoning causing deaths in developing countries costing them billions of U.S dollars in medical care, medical and social costs (Fratmico *et al*., 2005 and FDA, 2012).

The bacterial pathogens that are rather likely to be found in commonly slaughtered livestock (sheep, cattle, and swine) and poultry (chicken and turkey) include ***Salmonella* spp., *Escherichiae coli* and *Listeria monocytogenes*** .These pathogens have been implicated in widely publicized food-borne illnesses outbreaks associated with poultry products and meat consumption. Meat and meat products from retail outlets have been documented to be contaminated at different rates and implicated in infections and outbreaks associated with ***Salmonella* spp., *E.coli* and *L. monocytogenes*** and others (Kupradit *et al*., 2013; Rodpai *et al*., 2013; Saeed *et al*., 2013; Zhao *et al*., 2014; Adwan *et al*., 2015).

2.3. Bacterial Contamination of Chicken Meat:-

Chicken meat is considered to be a highly nutritive food with a relatively cheap price, low cholesterol content and fat, consumed worldwide. yet, it is highly perishable, and its storage life is relatively short even at refrigerated temperature (Mantilla *et al*., 2011 and Sheir *et al*., 2020).

Carrizosa and his team (2017) indicated that Chicken meat has a short shelf life because psychrotrophic bacteria causes off-flavors or spoilage even at cold temperature storage conditions. The spoilage of meat depends on the pH level, storage temperature, biodiversity of bacterial groups and availability of oxygen (Ercolini *et al*., 2010 and Sheir *et al*., 2020), also those factors, in turn, are associated closely with the growth of spoilage bacteria.

The misuse of temperature control and poor food handling could increase the growth of microorganisms which probably leads to the contamination and spoilage of

food (Gour *et al*., 2014), storage temperature, nevertheless, is the most important factors that affects the growth of bacteria existing in chicken meat.

In a study conducted on chickens, it was found that live chicken skin harbors bacteria at numbers close to $1500/cm^2$ which represented micro flora , while in the final product the bacterial number was more than $2500/cm^2$ and this may be due to many factors like legs, feathers, fecal material ,viscera ,boiling water, washing and skinning equipment (Ayres *et al*., 1980 and Al-jasser, 2012). One of the studies conducted by some researchers on chilled chicken, it was found that bacteria belonging to the genus ***Pseudomonas*** constitute more than a third of the bacteria found on chicken meat, followed by ***Acenitobacter***, ***Flavobacterium*** , ***Enterobacterium*** and ***Corynebacterium*** members. In the meat of chickens treated with antibiotics such as theroxytetracycline and chorotetracycline, it was found that yeasts are dominant, where bacteria are inhibited by antibiotics, leaving yeasts, especially ***Rhodotorula*** and ***Torulopsis***. As for the chilled chickens packed under vacuum, it was found that the members of the ***Enterobacter*** group dominate over ***Pseudomonas*** which the first facultative ones, while the second is aerobic, which does not grow under these conditions. (Asghar *et al.*,1988; Octavian and Aida, 2010; Al-jasser, 2012; Haleem *et al.*, 2013*)*.

Haleem and his colleagues (2013) showed that In Iraq, poultry is slaughtered manually, thus, contaminated by different types of microorganism like bacteria, fungus even parasites from soil or from contaminated earth with other poultry wastes. Sometimes washing is not useful to get rid of microorganisms and we need to cook immediately for meat to kill these microorganisms especially pathogenic one, while the frozen poultry meat and its product which comes to markets from unknown origins may be contaminated by certain microbial pollutants, due to unknown sources, processing and transportation in addition to the instability of electrical power.

2.4. Overview of Foodborne Illness:-

Foodborne pathogens usually refer to pathogenic bacteria presented within the process and circulation of food, which survive, develop and metabolize in food and

cause food spoilage and destruction. At the same time, a few pathogenic bacteria will excrete poisonous substances, directly or indirectly lead to sickness of individuals (Zhang *et al*., 2017). The most common microbial genera isolated from fresh meat are ***Acinetobacter, Enterobacteriaceae, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus, Micrococcus, lactic acid bacteria, and Pseudomonas*** (Doulgeraki *et al*., 2012).

The common pathogenic bacteria which will cause bacterial sickness are ***Escherichia*** (especially hemorrhagic ***Escherichia coli* O157:H7**), ***Salmonella, Shigella,* Pathogenic *Vibrio*** (***Vibrio cholerae*** and ***parahaemolyticus)***, ***Staphylococcus aureus***. In recent years, more and more microorganisms which may result in human food poisoning are found, and they were mainly ***listeria monocytogenes*** and ***Campylobacter jejuni*** (Gao *et al*., 2014).

Sun (2015) showed that food borne pathogens have led to several food recall or food safety incidents in recent years, including the ten food recall events in united states due to ***Listeria*** contamination.

According to WHO statistics, around1billion and 500 million cases of diarrhea happened annually around the world, resulting in the death of three million children under 5 years old, about 70% of which are caused by food borne pathogens contamination food.

The U.S. Centers for Disease Control and Prevention (CDC) estimated that every year within the United states about 32 million and 150 thousand patients are hospitalized by food borne pathogens ,and 5200 cases are dead. About 14 million cases of foodborne diseases were caused by known pathogens, among them, 60 thousand people were hospitalized and 1800 people died. The annual economic loss caused by food borne diseases can be reached to 2 billion and 600 million Australian dollars in Australia. It can be seen that food borne pathogens have caused an enormous and expanding world public health problem (Ju *et al*., 2019). The table (2-1) explain some pathogens microorganism responsible for food-borne illness as described by Velusamy *et al*., (2010) and FDA, (2020).

Table 2.1. Some pathogenic microorganisms responsible for food-borne illness (Velusamy *et al*., 2010) and FDA, 2020).

Pathogen	Associated foods (reported food contamination)	Onset time after ingestion	Duration	Name of the disease	Symptoms
Campylobacter jejuni	Raw milk, and Raw or under – cooked meat, poultry or shellfish	2-5 days	2-10 days	Campylobacteriosis	Fever, head ach, and muscle pain followed by diarrhea, abdominal pain and nausea
***Salmonella* spp.**	Raw/undercooked eggs, poultry, meat, raw milk, dairy product; sea food; chocolate; salat and spices	6-48 hours	4-7 days	Salmonellosis	Stomach pain, diarrhea, nausea, chills, fever, and headache
E. coli	Raw/undercooked eggs, poultry, meat; raw milk, dairy product, sea food; and leafy vegetables	1-3 days	3-7or more days	Hemorrhagic colitis	Stomach pain, diarrhea, nausea, chills, fever, and headache
***E.coli* O157:H7**	Under cooked beef(burger) unpasteurized milk, juice, water	1-8 days	5-10 days	Hemorrhagic colitis or *E.coli* O157:H7 infection	Severe bloody diarrhea, abdomen pain ,vomiting ,little or no fever caused kidney failure in younger
L.monocytogens	Soft cheese, raw milk, ice cream, vegetables, raw meat and poultry	9-48hfor gastrointestal	variable	Listeriosis	Fever, chills, headache, backache, sometimes abdominal pain and diarrhea
Bacillus cereus	Meats, milk vegetables Fish, rice, pasta	10-16 hours	24-48 hours	*Bacillus cereus* food poising	Diarrhea, abdominal cramps, nausea, and vomiting
Clostridium botulinum	canned foods, garlic in oil vacuum tightly wrapped food	12-72 hours	variable	Food borne botulism	Double vision, droopy eyelids trouble speaking, swallowing, difficulty breathing

Clostridium perfringens	Undercooked meats, meat products, and gravies	8-16 hours	Usually 24hours	Perfringens food poisoning	Abdominal cramps and diarrhea
Shigella	Salad, raw vegetables dairy product, and poultry	24-48 hours	4-7 days	Shigellosis	Abdominal pain, cramps fever, vomiting and diarrhea containing blood and mucus
Noroviruses	Raw product, contaminate drink water ,cooked ,uncooked foods ,shillfish	12-48 hours	12-60 hours	Viral gastroenteritis ,winter diarrhea, acute non gastroenteritis, food poising	Nausea, vomiting, abdominal cramping ,diarrhea ,fever, headache, diarrhea is more in adults ,vomiting is more in children
Vibrio parahaemolyicus	Raw, improperly cooked, or not cooked, contaminated fish ,shellfish, and oysters	4-96 hours	2-5 days	V. Parahaemolyticuse associated gastroenteritis	Diarrhea abdominal cramps, nausea, vomiting, headache fever and chills
Vibrio vulnificus	Raw or contaminated oysters, clams and crabs	1-7 days	2-8 days	Syndrome called primary septicemia	Diarrhea, and wound infection
Staphylococcus aureus	Unrefrigerated or improperly refrigerated meats ,potato ,egg, salads and cream pastries	1-6 hours	24-48 hours	Staphylococcal food poising	Sudden onset of severe nausea and vomiting. Abdominal cramps .diarrhea and fever may be present
Hepatitis A	Raw product, contaminated water ,cooked and uncooked foods ,shill fish	28 days average (15-50)days	Variable 2 weeks-3month	Hepatitis	Diarrhea ,dark urine ,jaundice and flu like symptoms I .e. fever ,headache ,nausea ,abdominal pain
Cryptosporidium	Various types of fresh products (imported berries,lettuce,basil)	2-10 days	May be remit and relapsing over weeks to months	Intestinal cryptosporidiosis	Diarrhea (watery)stomach cramps,upset stomach, slight fever

2.5. Food Spoilage and Food Pathogens:-

Food spoilage is defined as a change within the quality of food that renders it undesirable and unfit for consumption, either by humans or animals, because of spoilage indicators like changes in texture , appearance and objectionable odor (Blackburn, 2006; Nychas, and Panagou, 2011; Lianou *et al*., 2016). It's a complex process because of underlying causes that may be broadly grouped as microbiological, chemical, or physical (Petruzzi *et al.*, 2017). Despite the increasing technological progress in food science and technology over the years, spoilage of food remains a worldwide problem (Petruzzi *et al.*, 2017).

Snyder and Worobo (2018) Show that Food spoilage incurs huge economic losses to producers (farmers), retailers, and consumers. Microbes are the foremost common explanation for food spoilage and are ubiquitous, because they're too small to be seen with the human eye, aside from molds, colonization of exposed food by bacteria and yeasts may remain unnoticed (Hammond *et al*., 2015).

Colonization of food by spoilage microbes occurs in various ways, counting on the sort of food for examples food with high water content like meat, milk and seafood easily get spoiled by bacteria unlike food with low water content, while spoilage of dry food is sometimes initiated by molds or yeasts. However, the complexity of food spoilage and therefore the interconnectivity of things contributing to it make it difficult to resolve the problem(Odeyemi, 2020).

Microorganisms are available naturally within the surrounding environment; therefore, they'll easily reaching food during harvesting, slaughtering, processing, and packaging (Hatab *et al*., 2016). These microorganisms can survive under adverse conditions utilized in the food preservation like low temperature, modified atmosphere packaging, vacuum packaging, also as resist conventional pasteurization (Dimitrijević *et al*., 2007; Provincial *et al*., 2013; Saraiva *et al*., 2016; Säde *et al*., 2017).

There are about 200 known food-borne pathogens within the world. Unfortunately, these organisms can't be seen, smelled, or tasted ,it often takes only a few of them to infect a person . Meat is among the most perishable foods and a good environment for

the replication of spoilage microorganisms thanks to its high concentrations of nutrients and high-water activity (Húngaro *et al*., 2016).

Boziaris and Parlapani (2017) affirm that spoilage is because of the so-called specific spoilage organisms, which predominate and form metabolites and thereby alter the organoleptic characteristics of meat and these making it inappropriate for consumption. This, within the belief of Nattress *et al*., (2001) that it caused substantial economic losses for both producers and retailers. During slaughterhouse processing, carcasses may be contaminated by the skin, feces, water, intestinal content, personnel and processing room equipment.

2.6. Effects of Environmental Conditions on Proliferation of Meat Spoilage Microorganisms:-

2.6.1. Temperature:-

Storage temperature and period are crucial for spoilage of meat and meat products, Since the temperature of the refrigerated storage, from the point of view of the researcher Casaburi *et al*., (2015) impacts the selection of psychrotrophic microorganisms.

One of the primary organisms that replicated in vacuum-packed lamb stored at –1.2 °C were ***Carnobacterium, Yersinia, and Clostridium*** **spp**., whereas at 8°C, ***Hafnia, Lactococcus, and Providencia*** **spp**. were also observed (Kaur *et al*., 2017).

Borch *et al*., (1996) and Odeyemi *et al*., (2020) provides an explanation for that reduction of bacterial growth and alteration of microbial groups in vacuum-packed beef at storage temperature. Total possible counts attained 10^7 cfu/g after 14 weeks at 1.5 °C while at 4 °C the equal counts were attain after three weeks. On the contrary, the counts of bacteria inducing spoilage increased in aerobically stored chilled meat. In beef stored aerobically at 5 °C for 7 days, Russo *et al*., (2006) gave proof for increased ***B. thermosphacta*** (from 2.5×10^4 to 3.4×10^6cfu/g), ***Pseudomonas*** **spp.**

(from 5.0 $\times 10^4$ to 1.0 $\times 10^5$ cfu/g), ***Enterobacteriaceae*** (from 2.0 $\times 10^3$to 1.1 $\times 10^5$ cfu/g), and **lactic acid bacteria** (from 3.0 $\times 10^3$ to 3.4 $\times 10^6$ cfu/g) counts.

Kaur *et al*., (2017) encouraged the usage of the synergic effect of the modified environment or vacuum packaging, pH, cold storage and antimicrobial bacterial activity to slow down meat spoilage.

Accordingly, the temperature at which food is preserved determines the nature and numbers of microorganisms that grow in it. Preserving food at low temperatures leads to a result of a temperature lower than the optimum temperature for growth, which causes a rapid decrease in the metabolic activity of microorganisms due to the low rate of biochemical reactions. The viscosity of cellular fluids increases, and the lipids of the cell membrane harden, and if this decrease continues significantly, cellular growth stops completely (Jay *et al*., 2008).

2.6.2. PH :-

After the animal slaughter process is completed, changes in the acidity of the meat occur which causes a decrease in the pH of the meat from (5.4 to 5.8). In animals that suffered from stress before being slaughtered, the dark firm and dry meat phenomenon, that is accompanied by high meat pH could be observed. It was proven that bacterial nutrients in meat with high pH had been reduced. Such meat undergoes spoilage in shorter terms as microorganisms rapidly attack and hydrolyze amino acids (Borch *et al*., 1996 and Odeyemi *et al*., 2020).

There is a substantial positive correlation among meat pH and meat bacterial counts (Alonso-Calleja *et al*., 2004). As the high meat pH ends in rapid spoilage because of extensive bacterial replication and consumption of nutrients in meat .Wang and his team, (2017) reported that **lactic acid bacteria** produce lactic acid which barely decreases pH while in their absence meat pH is insignificantly higher at the same time as most ***pseudomonads*** produce a metalloproteinase with pH optimum among 6.5 and 8.

2.6.3. Water Activity, Moisture, and Salt Content :-

Water activity is an important indicator for identifying the speed of food spoilage. The values of the water activity range from zero to one, in fresh food the water activity is close to the number one, and therefore it is easy to spoil it due to the rapid growth of microorganisms in it, unlike dry food which have lower limit of water activity that do not allow the growth of microorganisms (Jay *et al.*, 2008).

Sodium chloride is among the most usually used additives in meat industry because of its low cost and numerous properties. It is preserving and antimicrobial agent because it reduces the water activity of foods. Also, the addition of salt to meat products affects some enzymes and thus improves the water preserving capacity and flavor of meat (Mariutti and Bragagnolo, 2017).

Odeyemi *et al.*, (2020) reported that the addition of 4% salt (sodium chloride) to meat products decreased their water activity from 0.99 to 0.97 preventing ***Pseudomonas*** and **Enterobacteriaceae** growth, however **lactic acid bacteria** and **yeasts** replicated. Nevertheless, the growth rate and lag phase of lactic acid bacteria were additionally influenced by reduced water activity. For instance, as water activity decreased from 0.98 to 0.96 in sausages the lag phase of lactobacilli increased 3 times even as the growth rate marked a twofold reduction (Plavsic *et al.*, 2015).

2.6.4. Packaging Atmosphere :-

Packaging is another vital aspect determining the microorganisms that will reproduce and spread in fresh meat. Therefore, the spoilage is because of bacteria that are initially found in meat. Casaburi *et al.*, (2015) and Dohlen *et al.*, (2017) support the thesis that the presence or absence of oxygen has a strong effect on the variety of microbial species replicating in meat. That means different microbial species will develop relying on whether or not meat is packed in aerobic or anaerobic conditions or in a modified atmosphere.

According to Kaur and his team (2017) the packaging used for vacuum or modified atmosphere packaging are badly permeable to oxygen and thus, inhibit the growth of

aerobic spoilage microorganisms and consequently, increase the shelf-life of the product. Also, the modified atmosphere includes gases at variable ratios for example, 70% O2 and 30% CO2 (Säde *et al*., 2017) 80% O2 and 20% CO2, and 65% N2 and 35% CO2 (Höll *et al*., 2016), which similarly inhibit spoilage microorganisms in meat.

Nychas *et al*. (2008) suggested that in most cases, ***Pseudomona*s spp**. had been responsible for the spoilage of aerobically spoiled meat. Lactic acid bacteria predominated in vacuum-packed meat (Nattress *et al*., 2001). Säde *et al*., (2017) isolated ***Carnobacterium***, ***Brochothrix***, ***Leuconosto***, and ***Lactococcus*** from beef packed in modified atmosphere.

2.7. Psychrophilic and Psychrotrophic Bacteria :-

Hypothermic microorganisms can be defined as those that have the ability to grow and multiply at temperatures below 20°C, including psychrophilic and psychrotrophic microorganisms . Psychrophilic microorganisms have a maximum growth temperature at 20°C or below and it is restricted to permanently cold habitats while Psychrotrophic microorganisms have a maximum growth temperature above 20°C but have ability to growth in low temperature and it is widespread in foods and in natural environments (Gounot, 1986 and Wei *et al*., 2019).

The cooling of most foods at low temperature does not protect them from spoilage but only reduce the rate of food deterioration (Nychas *et al*., 2008). There are many kinds of microorganisms can be growth in the refrigeration condition like yeast ,bacteria and fungi are mainly found in the chilling foods (Wei *et al*., 2019).

The growth of Psychrophile and Psychrotrophic bacteria in low temperature due to they have fat cell membranes and possess chemical resistance to cooling and often produce proteins called "Cold shock protein" to protect the interior liquid and its DNA (Rapuntean, 2005).

Psychrotrophic bacteria can be divided into two types : Gram-positive bacteria including ***Corynebaterium, Streptococcus, Bacillus, Clostridium, Listeria, Lactobacillus, Paenibacillus*** and ***Microbacterium*** and Gram-negative bacteria such as

Pseudomonas, Flarobacterium, Alcaligenes, Achromobacter, , Aeromonas, Yersinia and ***Chromobacterium*** (Hantsiszacharov *et al.*, 2007).

Some types of bacteria have a special mechanism that enables them to grow at low temperatures which depends on several features , including : cell membranes contain lipid which helps them to absorb of nutrients in the environment under low temperature conditions (Wei *et al.*, 2019). Further, psychrotrophic bacteria can excrete large quantities of extracellular proteases and lipases to decompose biological macromolecules into small molecules under the condition of low temperature to ensure the nutritional needs (Jaouen *et al.*, 2004).

Ernst *et al.*, (2018) confirmed that the posttranscriptional modifications in tRNA of these bacteria are known to have many functions, inclusive maintaining tertiary structures and cellular adaptation to environmental factors.

Enzymes activity is another important features that are affecting on the growth of bacteria, which is inhibited when the enzyme activity is affected at low temperatures. So, psychrotrophic bacteria could be grow at low temperature with the cold-adapted enzymes, which have a higher catalytic activity in low temperatures (Ke *et al.*, 2018).Moreover, psychrotrophic bacteria can also increase the enzyme production to recompense for the lack of enzyme catalytic reaction rate (Marx *et al.*, 2007).

Cold shock proteins "Csp" are found in a wide range of psychrotrophic bacteria (Newkirk *et al.*, 1994 and Lee *et al.*, 2014). Usually, the structures of cold shock protein have two highly conserved nucleic acid-binding motifs: Ribonucleoprotein "RNP" 1 and 2 (Caruso *et al.*, 2017).

Some researchers explained that when the ambient temperature is lower than the growth support temperature, the effect of "Csp" can be expressed, so it's binding to the single stranded nucleic acids and acting as an RNA chaperone to regulate translation .The production of cold shock proteins is certainly a strategy for psychrotrophic bacteria to adaptation to a low- temperature environment (Wei *et al.*, 2019).

2.8. Meat Preservation:-

Meat can be stored safely for lengthy periods by following the suitable hygienic. The reason of meat preservation is in preventing contamination and delay of spoilage by microbial enzymes and protecting enzymes of meat from damaging. Meat preservation relies upon on procedures and processes to manage microbial growth and the impact of its enzymes on meat (Ledward, 2003 and Rajendran *et al*., 2019).

Stephen (2020) explained that the aim of food preservation operations to prolong the food's shelf life for as long as possible, by using all the various scientific means in the correct ways in order to prevent the natural spoilage of food by controlling microorganisms in food through: reducing or preventing microorganisms from reaching food, removing or delaying and impeding the growth of microorganisms in food, killing and exterminating microorganisms in food, so that the properties of a natural, chemical and nutritional substance determine the best way to preserve it which no negative impact on the nutritional value of the final product. There are many methods used to preserved meat and meat product (Rajendran *et al*., 2019).

- **Thermal Methods** like(Smoking, Canning, Drying , Hot smoking and Cold smoking
- **Non -Thermal Methods** like (Freezing, Freeze-drying , Chilling and adding Salt i.e. curing meat).
- **Nitrates & Nitrites**.
- **Fermentation & Pickling.**
- **Irradiation.**
- **Hydrostatic Pressure Processing.**
- **Hydrodynamic Pressure Processing**

2.8.1. Preservation of Meat by Freezing Method:

This is one of the best methods of preservation of foodstuffs which includes all varieties of meat and this method preserve meat like natural state where it is slowing down of the enzymatic reactions and growth of microbes. But the organisms will be

deactivated rather than killed and in all likelihood, could be activated once the frozen product is thawed (Rajendran *et al*., 2019).

Sohaib *et al*., (2016) they refered that microorganisms such as bacteria and yeast grow well at specific temperatures normally between (4.4°C -60°C). By decreasing the temperature approximately under freezing condition that is below (4°C) their multiplication will substantially be arrested without killing them and making them slow the spoilage. A minimum storage temperature of no less than (-12°C) is vital for longer shelf life with desired characteristics without losing flavor. But this could be possible only with the availability of a freezer and reliable electricity uninterrupted power supply.

The meat or cut portions need to be wrapped with freezer bags or paper to prevent freezer burn and also to ensure for air tightness to keep away from contamination. Uncooked meat such as steaks or chops can be frozen safely for (4-12) months and further uncooked ground meat also should safely be stored for (3-4)months while the cooked meat can be stored for(2-3) months. The poultry meat of both cooked and uncooked may be stored for (3-12) months. It is important to fill the cooler with ice on the bottom and then place meat and then cover with extra ice for the good meat preservation and making sure that meat is surrounded by ice to ensure complete and uniform freezing (Rajendran *et al*., 2019).

All the foods which includes meat begins to freeze at specific temperatures about (– 6°C) depending upon their cellular composition. If the food is slowly frozen, the ice crystal formed can be larger which cause the rupture of cells and the destruction of texture in meat, fish, vegetables, and fruits. To overcome this problem, the approach of quick-freezing has been developed in which a food is cooled under its freezing point as quickly as possible. The final product obtained tends to have a firm and natural texture than the slow frozen technology when it's thawed. It is vital to maintain at temperature at or under(-12°C) in case of the product needs to have good shelf life without losing its flavor . This technology may change the texture of most of the fruits

and vegetables however meats and fishes are doing well for this technology (Rahman *et al.*, 2007 and Rajendran *et al.*, 2019).

2.9:Diagnostic Methods for Enumeration and Detection of Frozen Food bacteria:-

2.9.1: Traditional Methods:-

Traditional detection ways depend on selective cultivation techniques concerted with standard biochemical identifications. These methods have the advantage of being a waste of time and repeated attempt, sampling and enumeration by mistake, because pathogenic bacteria can be present in small numbers. In fact, the low throughput of these traditional methods does not allow rapid screening of large numbers of food samples for one or more pathogens (Abubakar *et al.*, 2007 and Chen *et al.*, 2012).

Many bacterial species found in frozen meat ,meat and chicken products have been studied and discovered ,which have cause the spoilage food or foodborne diseases and their resistance to antibiotics from this species such as ***Staphylococcous*** **spp. ,** ***Alcaligenes*** **spp. ,*Klebsiella*** **spp. ,*Enterococcus*** **spp. ,*Actinobacillus*** **spp. ,*Proteus*** **spp.**(Sultana *et al.*, 2014).

Some researchers studied the bacterial contamination in frozen beef meat product by using biochemical test like API Strips and Automated Vitek2 Compact System and they found the most important species were ***Lactobacillus*** **spp.,** ***Kocuria kristinae* , *Proteus mirabilis, Klebsiella pneumonia, Bacillus badius, Enterobacter gergoviae, Bacillus licheniformis*** (Ahmed and Sabiel, 2016) ,while the others studied the most important species of bacteria in poultry meat like Haleem *et al.* (2013) they found ***Pseudomonas, E.coli,*** **fecal** ***Streptococcus, Staphylococcus, Salmonella,*** **fecal coliform .**

2.9.2: Molecular Methods :-

2.9.2.1: Conventional Polymerase Chain Reaction (Monoplex PCR) :-

PCR is the most famous and confirmed nucleic acid amplification technique for detection of pathogenic microorganisms as mentioned by DeCory *et al.*, (2005) and

Zhao *et al*., (2014). The double-stranded DNA in this method was denature into single strand and the specific primers or single-stranded oligonucleotides anneal to the DNA strands ,and then followed by extension of the primers complementary to the single stranded DNA, with a thermostable DNA polymerase. These steps are frequent, and resulting in doubling of the initial numbers of target sequences with every cycle. This quantum of products of amplification can be imaged as a bundles on an ethidium-bromide stained electrophoresis gel (Zhao *et al*., 2014).

Identification based PCR amplification of target genes by sequencing is considered to be a dependable technique when completely developed and validated for a certain species. With the special advantages of rapidity, sensitivity, specificity, and less samples over culture-based methods, much of PCR assays for the detection and validation of foodborne bacteria and viruses in food have been progressing and applied in food samples (Ikeda *et al*., 2007).

In the last years, rapid nucleic acid amplification and detection technologies have been continuously applied to pathogen recognition in food industry. Newly, the real-time polymerase chain reaction has successfully been perfect to identify pathogens in different food products. Whilst , these methods are not only very costly for routine use in common testing laboratories, limited to two or three different types of pathogenic bacteria per detection assay (Wang *et al*., 2007; Elizaquı´vel and Aznar 2008; Suo *et al*., 2010).

2.9.2.2:Multiplex Polymerase Chain Reaction (m-PCR):-

Some researchers have relied on molecular diagnostics by using PCR ,Multiplex PCR or Nested PCR methods for detection of foodborne pathogens such as ***Vibrio cholera*** **,** ***E. coli*** **O157:H7** **,** ***Salmonella*** **spp.** **,** ***Vibrio parahaemolyticus, Staphylococcus aureus and Listeria monocytogenes*** (Lei *et al*., 2008).

Kawasaki *et al*., (2009) they detect ***Salmonella*** **spp.,** ***Listeria monocytogenes, and Escherichia coli*** **O157:H7** in foods and in food subjected to freezing by using

Multiplex PCR System , as well as Elbasheir *et al.*, (2019) were isolated of 30 bacteria obtained from swabs of 30 fresh chickens carcasses.

The simultaneous amplification of more than one locus is wanted for a fast detection of multiple microorganisms in a single reaction. It is a methodology express it as multiplex PCR (mPCR), where the sundry specific primer sets are combined into a single PCR test (Chamberlain *et al.* 1988 and Zhao *et al.*, 2014) .Clearly, the primers design is a key factor in the development of a multiplex PCR assay. May be there some interaction between the multiple primer sets, so the primer concentrations may be adjusted in order to produce reliable yields of all the PCR products. while, the primer sets should be prepared with a similar annealing temperature, whereas providing a method to distinguish between amplicons following thermal cycling.

Now a day, m PCR can also be beneficial to define the structure of confirmed microbial communities and to estimate the community dynamics, during fermentation or in response to environmental variations(Kong *et al.*, 2002 ; Kupradit *et al.*, 2013; Zarei *et al.*, 2013).

Multiplex PCR jointly detecting sundry pathogens in a single-tube reaction has the possibility of provision time and tension , reducing testing related laboratory costs (Perry *et al.*, 2007 and Chen *et al.*, 2012).

Effective detection techniques are a prerequisite for the invention and identification of pathogenic bacteria in foods, food sources, and food processing plants. Because of the conventional culture method for detecting pathogens (Figure 2.1, left side) is time consumption, results are extremely not available while the food has been either released to the markets or consumed, this one increasing the risk of transmission of pathogens. Pathogens are oftentimes present in very low numbers against a background of native microflora, requisition the recovery of target organisms difficult (Kawasaki *et al.*, 2011)

Sensitive and rapid assays with high specificity are wanted for the detection of pathogenic bacteria in foods and another types of samples. PCR-based methods have the possibility for the rapid and sensitive detection of food-borne pathogens (Figure

2.1, right side). As a result of PCR the target unique genetic sequences, like the virulence genes of microorganisms, it has the advantage of being in the extreme specific assay. The multiplex PCR method has ability to determination the presence of ***E. coli , Salmonella* spp., *and L. monocytogenes*** directly from enrichment cultures via targeting the specific DNA sequences of every pathogen (Kawasaki *et al*., 2005 and Kawasaki *et al*., 2010) .

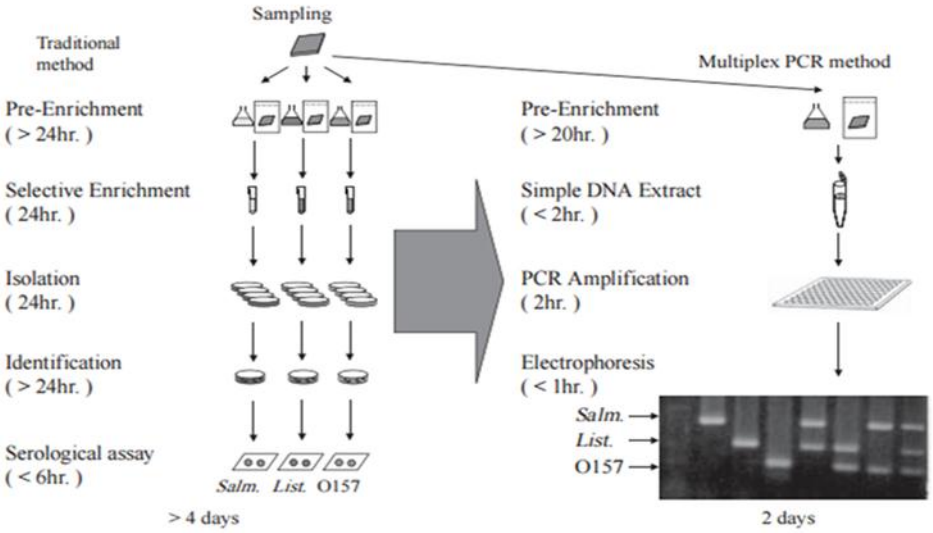


Figure 2.1 Schematic representation of detection procedure of conventional culture method and multiplex PCR method (Kawasaki *et al*., 2011).

2.9.3: 16 S r RNA Sequencing:-

Ribosomal RNA sequences and specially the 16S r RNA represented the most important current targets of study in bacterial evolution and ecology. Including the determination of phylogenetic relation among taxa , the exploration of bacterial diversity in the environment and the quantification of the relative abundance of taxa of different ranks (Hugenholtz *et al*., 1998 ; Větrovský and Baldrian, 2013).

Woo and his team (2000) and Suardana (2014) showed that after the invention of polymerase chain reaction (PCR) and automated DNA sequencing, the genome of some bacteria had been sequenced completely. Comparison of the genomic sequences of bacterial species appear that the 16S ribosomal RNA gene is highly conserved within a species , among species of the same genus . So, could be used as the new gold standard for the specification of bacteria.

For the study of bacterial phylogeny and taxonomy, 16S rRNA gene sequences are very helpful . The gene presence almost all bacteria, often existing as a multigene family or operons, the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time and the 16S rRNA gene (1500 bp) is large enough for informatics purposes (Patel , 2001).

CHAPTER THREE

MATERIALS & METHODS

3. Materials and Methods:-

3.1. Materials:-

3.1.1: Apparatuses and Tools:-

Apparatuses and tools used in this study are summarized in table (3-1).

Table (3-1): Apparatuses and tools that used in this study.

N0.	Apparatus and Tool	Company/Origin
1	Autoclave	Hirayama/ Japan
2	Beakers	Iso Lab/Germnay
3	Burner	Indiamart/India
4	Biosafety Cabinet	Lab Tech/France
5	Colony counter	Boeco/Germany
6	Cooling Centrifuge	Eppendrof/Germany
7	Cylinder	Iso Lab /Germnay
8	Disposable Petri dishes	Al- Hani company/Lebenon
9	Electrophoresis apparatus	Consort/Belgium
10	Eppendrof tubes	Bioneer /South Kourea
11	Flask (250,500,1000)	Iso Lab/Germnay
12	Gel Documentation	Vilber lourmat/France
13	Gloves	Broche/Malaysia
14	Incubator	Human Lab/Korea
15	light Microscope	Olymps/Japan
16	Micropipettes	DragonMED/China
17	Oven	Memmert/Germnay
18	Refrigerator	Vistal/Poland
19	Sensitive Balance	Sartorius/Germnay

20	Slides and coveslides	Superestar/India
21	Spectrophotometer	Shimadzu/India
22	Standard wire loop	John Bolten/England
23	Test tubes	AFCO-Dispo/Jordan
24	Tips	Sterellin Ltd./UK
25	Thermal cycler apparatus	Prime /UK
26	U.V -Transilluminator	Electrofor /Italy
27	Vortex mixture	Medilab/South Korea
28	Vitek2 compact system	Biomerieux/ France
29	Water path	Memmert/Germnay

3.1.2 : Chemical and Biological materials :-

The chemical and biological materials that used in current study in table (3-2).

Table (3-2): Chemical and Biological materials used in the present study.

No.	Material	Company /origin
1	Absolute Ethanol	Scharlau /Spain
2	Agarose	Biobasic / Canada
3	50X TBE (Tris- Boric acid EDTA)	
4	DNA Ladder (100bp)	Bioneer / South Korea
5	Ethidium bromide	Promega / USA
6	Free water-Nuclease	

3.1.3 : Culture Media :-

The culture media which used in the present study are listed in the table(3-3).

Table (3-3) : Culture media which used in the present study.

No.	Media	Company/ Origin
1	Blood agar base	Himedia(India)
2	MacConkey agar	
3	Mannitol Salt agar	
4	Nutreint agar	
5	Nutreint broth	
6	Peptone water	Bio lab (India)
7	Sorbitol MacConkey agar	Oxoid (UK)
8	Thiosulfate Citrate Bile Sucrose Cholera (T.C.B.S)	

3.1.4 : The Kits :-

The kits which used in the current study are listed in the table (3-4).

Table (3-4) : The kits which used in the present study.

No.	Kit	Purpose	Company /Origins
1	Gram stain	Differentiation of shape and strain of bacteria	Himedia /India
2	Presto ™ Mini g DNA Bacteria	genomic Extraction bacterial DNA	Geneaid /Taiwan
3	Vitek 2-GP Kit	Identification of Gram Positive bacteria	Bio Merieux France
4	Vitek 2-GN Kit	Identification of Gram Negative bacteria	
5	AccuPower® PCR PriMix	primer by For amplified PCR	Bioneer/South

6	AccuPower® PCR MultiMix	For amplified Special primer by PCR	Korea

3.1.5 : The primers:-

The primers used in this study were prepared by Bioneer company (South Korea) as described by Miyoshi *et al.*, (2005) for universal primer and by Lei *et al.*, (2008) for special primers as summarized in table (3-5).

Table (3-5) : The primers which used in the present study.

No.	Bacteria	Target genes	Primers sequencing 5' → 3'	PCR Product size(bp)
1	All bacteria (universal)	16S rRNA	27 F 5´- AGAGTTTGATCCTGGCTCAG 3´ 1492 R 5´- GGTTACCTTGTTACGACTT 3´	1500
2	*Vibrio cholerae*	ctxAB	TGA AAT AAA GCA GTC AGG TG GGT ATT CTG CAC ACA AAT CAG	777
3	*E. coli* O157:H7	stx	TGG GTT TTT CTT CGG TAT CC CCAGTTCAG AGT GAG GTC CA	632
4	*Salmonella* spp.	invA	TAC TTA ACA GTG CTC GTT TAC ATA AAC TTC ATC GCA CCG TCA	570
5	*Vibrio* parahaemolyticus	tlh	CGG ATT ATG CAG AAG CAC TG ACT TTC TAG CAT TTT CTC TGC	444
6	*Staphylococcus aureus*	nuc	GCG ATT GAT GGT GAT ACG GTT AGC CAA GCC TTG ACG AAC TAA AGC	270
7	*Listeria monocytogenes*	hly	GCA TCT GCA TTC AAT AAA GA TGT CAC TGC ATC TCC GTG GT	174

3.2 : Methods :

The general steps of the current study are shown in figure (3-1).

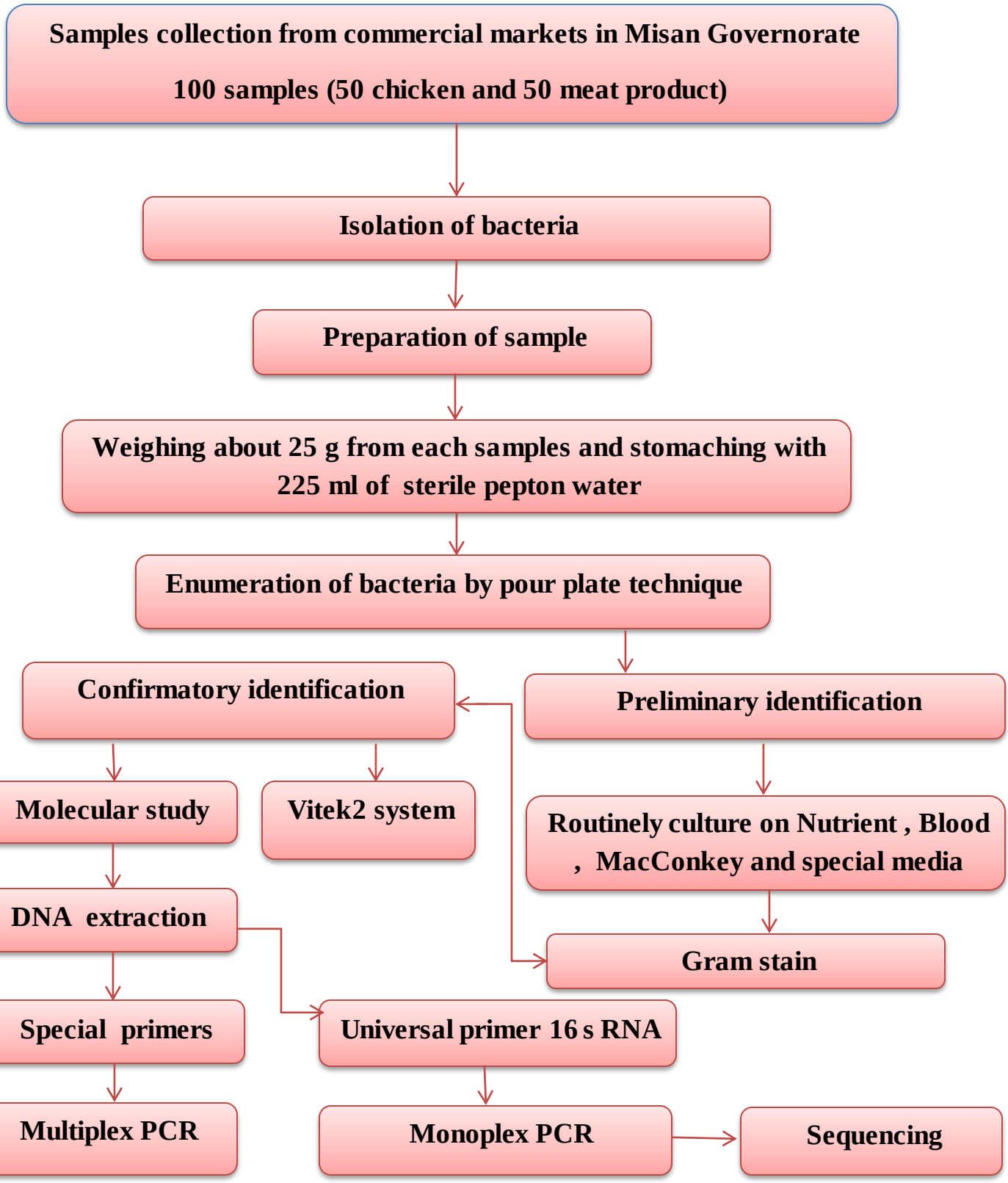


Figure (3-1) : The most important steps in the present study.

3.2.1 : Samples Collection :-

In this study,100 samples of frozen food were collected randomly from the markets of Misan governorate which included:

1- Meat products ,represented by (burger – sausage – kebab – shawarma -minced meat) where 10 samples for each product were collected .

2- Chicken products ,represented by (chest – thigh- liver- burger- kebab) where 10 samples for each product were collected .

-The samples included local and imported companies .

3.2.2. Preparation of Culture Media :-

All media were prepared according to the manufacturing companies instructions; they were brought to boil in water bath to dissolve all constituents completely, and then sterilized by autoclaving at 121°C for 15 min , otherwise the media were incubated at 37°C for 24h to ensure sterility .The type and the purpose of media used in the current study listed in the table (3-6).

Table (3-6): The media used in the current study and their purpose .

No.	Medium	The purpose of used
1-	Nutrient agar	used for the primary isolation and studying the cultural and phenotypic properties of the isolated bacteria.
2-	Blood agar	detect the ability of bacterial isolates to produce the hemolysin .
3-	MacConkey agar	diagnosing lactose-fermented bacteria.
4-	Sorbitol MacConkey agar	used to distinguish the pathogenic *E. coli* O157:H7 which unable to ferment sorbitol from nonpathogenic *E.coli* which ferment it .
5-	Mannitol Salt agar	used for identification of *Staphylococci*

6-	TCBS Cholera	used for isolation and identification of *Vibrio* spp.
7-	peptone water	used in primary enrichment of growth of bacteria .

3.2.3: Sterilization Methods:-

3.2.3.1: Sterilization by Dry Heat :-

The glassware was sterilized by oven at 150 °C for two and a half hours.

3.2.3.2: Sterilization by Autoclaving:-

The culture media were sterilized by autoclaving at 121℃ for 15 minutes under pressure 15 psi.

3.2.4: Isolation of Bacteria from Frozen Food Samples:-

The procedures of Khalafallah *et al*., (2020) and Hafez *et al*., (2019) were used for the isolation of bacteria from frozen food samples with some modification :

The samples were collected and transferred directly to the microbiology laboratory at the collage of science under aseptic conditions to start working on them while they are in the frozen state .

Twenty five grams of each examined samples were weighed by sterile sensitive balance where they were taken from different parts of the samples tested then, placed into sterile flasks to which, 225 ml of 0.1% sterile buffered peptone water were aseptically added to the content of flasks. Thereafter, each sample was homogenized in a blender at 2000 rpm for 1-2 minutes to provide a homogenate of 1/10 dilution. One ml from the original dilution was transferred by sterile pipette to the first sterile tube having 9ml sterile buffered peptone water 0.1% (10^{-1}) , after mixing well, one milliliter was taken from the same tube (10^{-1}) transferred to the second tube (10^{-2}) and this process continued sequentially until reaching dilution (10^{-10}).

After completing the serial dilutions, 0.1 ml of the dilution 10^{-10} was taken and deployed by sterilized swabs on the surface of the nutrient agar. The plates were inverted and incubated at 37 º C for 24 hours.

After 24 hours growing colonies on the nutrient agar were counted by colony counter. The CFU/ml was calculated :

CFU per ml = No. of colonies * dilution factor/ volume of inoculum

Finally, the bacterial isolates were purified by making several subcultures of the colonies, to be ready for morphological, biochemical and molecular tests .

3.2.5: Identification of Bacteria:-

3.2.5.1: Conventional Tests:-

The growing colonies of bacterial species isolates were initially identified depending on:

3.2.5.1.1: Morphological Characterization of Bacteria :-

The morphological characteristics of the growing colonies of bacteria include color, size, form, elevation and margin of the colonies on ordinary, enrichment, selective and differential media (Nutrient agar, Blood agar, MacConkey agar, Mannitol salt agar, MacConkey sorbitol agar and TCBS agar) and incubated as concerning with the certain bacteria (Goldmann and Lorrence, 2009).

3.2.5.1.2: Gram Staining:-

Gram stain were used to differentiate shapes of bacterial isolates and to distinguish between Gram positive and Gram negative bacteria (Beveridge, 2001) .

3.2.5.1.3: Identification by Vitek2 Compact System:-

In current study the Vitek2 system was used in order to confirm the diagnosis of bacterial isolates from Frozen food. The system requires a bacterial suspension from

the suspected bacteria, which placed in the inoculated tube and after that the suspension was transferred to the card, which is incubated in thermally controlled conditions. As a result of the metabolic activity of the bacteria the colors changes in the card, and every 15 minutes are measured in interrupted form by light intensity. Finally, the information stored, analyzed and printed automatically (Pincus, 2006).

The steps are described in more detail as follows :

I- Preparation of Bacterial Suspension :

A suitable number of bacterial colonies are transferred by a sterile disposable loop from pure culture which grown on MacConkey agar, blood agar or nutrient agar, and suspended in sterile saline solution 0.45% (3 ml) in a clear plastic test tube. The density of suspension was checked after adjusted the turbidity by turbidity meter (densichek) at the range (0.5- 0.63).

II- Inoculation of Identification Card :

Apparatus of integrated vacuum was used for inoculated identification card with bacterial suspension ,the cassette (special rack) was placed in test tube containing the bacterial suspension in the neighboring slot the identification card was placed. While inserted into the corresponding suspension tube(transfer tube). The cassette can accommodate up to 10 tests or up to 15 tests. After that, the filled cassette placed either transported automatically or manually into a vacuum chamber station. After, the air reintroduced and vacuum was applied into the station, the bacterial suspension inserted and forced through the transfer tube into microchannels which that filled all the test wells.

III- Card Sealing and Incubation :

A mechanism was used to passed an inoculated card which cuts off the transfer tube and then locks the card before it is loaded into the carousel incubator. The carousel incubator can hold up to 30 or up to 60 cards, all card kinds were incubated on-line at (

35.5 + 1.0º C). Each card is transferred once every(15 minutes) from the carousel incubator, transported for reaction reading by the optical system. Then returned to the carousel incubator until the next reading time. Data collected during the entire incubation period at (15 minutes) intervals.

3.2.5.1.4: Methods of Short and Long -Term Preservation:-

Nutrient agar was distributed in screw cap tubes in 20 ml quantities sterilized and left slant to solidify then it is incubated for 24 hours to ensure that it is free of contamination ,the inclined surface was inoculated with bacteria by screening then incubated at 37º C for 24 h and stored at 4ºC.

The bacteria were transferred to a new medium, each month to activate the isolates and avoid contamination (Collee *et al.*, 1996). For long preservation, Nutrient broth supplemented with 15%. glycerol had been used to frozen at temperature(-20 ºC) for several months or year (Collee *et al.*, 1996).

3.2.5.2: Molecular Identification:-

For molecular detection of some genes of bacteria isolated from frozen food, Polymerase Chain Reaction (PCR) assay was performed as following :

3.2.5.2.1: Genomic DNA Extraction:-

Genomic DNA Mini Bacteria Kit was used to extract Genomic DNA from bacteria according to the company's instructions, as follows :-

Step 1: Sample Preparation:-

• One milliliters of fresh culture(pure colonies) was added to a 1.5ml of micro centrifuge tube.

•The micro centrifuge tube was centrifuged for 1 minute at 14,000 rpm. ,then the supernatant was discard.

• One hundred and eighty microliters of GT buffer were added by micropipette. Then the cell pellet was re suspended by the vortex apparatus.

•Twenty microliters of proteinase K (should be dilution by added of distilled water) was added and incubated for 10 minutes at 60^{o} C in the incubator , the tubes were inverted during incubation every 3minutes.

Step 2: Lysis Step:-

∗Two hundred microliters of GB buffer was added to the sample and mixed well for 10 seconds by vortex .

∗The tubes was incubated at 60^{o} C for at less 10 minutes to ensure the sample lysate is clear, the tubes was inverted every 3 minutes during incubation. At this time the Elution buffer was pre-heated (200µl per sample) to 70^{o} C (for step DNA Elution) by used water bath apparatus.

Step 3: DNA Binding:-

◊Two hundred microliters of (absolute ethanol) was added to the sample lysate and mixed immediately by shaking vigorously.

◊Two milliliters of GD column was placed in a collection tube.

◊ The mixture (including any insoluble precipitate) was transferred to GD column then the mixture was centrifuged for 2 minutes at 14,000 rpm.

◊Two milliliters of the collection tubes containing the flow-through were discard.

◊The GD column was placed in two ml a new collection tube.

Step 4: Washing Step:-

- Four hundred microliters of (W1 Buffer) was added to the GD column and then centrifuge for 30 seconds at 14,000 rpm then discard the flow through ,the GD column were placed back in two ml collection tube.

- Six hundred microliters of wash buffer (make sure added of ethanol) was added to the GD column, then centrifuge for 30 second at 14,000 rpm after that discard the flow through, the GD column were placed back in two ml collection tube .
- The columns matrix were centrifuge for 3 minutes at 14,000 rpm to dry.
- The dried GD column was transferred to 1.5ml a clean micro centrifuge tube.

Step 5:Elution:-

•The preheated(Elution Buffer) was added into the center of the column matrix.

•The tubes were left at least 3 minutes to allow Elution buffer to be completely absorbed.

• The tubes was placed in Centrifuge for 30 seconds at 14,000 rpm to elute the purified DNA which was stored at 2-8º C.

3.2.5.2.2: Detection of DNA Content by Agarose Gel Electrophoresis:-

Agarose gel was prepared according to Sambrook and Russell (2006) with some modification used to confirm the integrity and presence of extracted DNA of bacterial isolates and performed as following:-

1- One hundred of(1X TBE buffer) was taken in a flask.

2-One gram of agarose powder was added to 100 ml of (1X TBE buffer).

3-The solution was heated up to boiling point by using a microwave until all the gel particles were dissolved.

4- Four microliters of ethidium bromide (0.5 µg/ml) was added to the agarose solution, and then stirred the agarose in order to get mixing.

5-The solution was left to cool at the room temperature.

6- The agarose solution was poured into the gel tray, and fixing the comb from one edge in 1 cm away .

7- The agarose was left until solidify for 30 minutes at room temperature. After that the fixed comb was removed carefully and the gel tray was placed in the gel tank. The tank was filled with 1X TBE buffer, until the buffer covered (3-5 mm) the surface of the gel.

8- Five microliters of DNA sample was transferred to Eppendrof tube and 2µl of loading dye was added to the tube and mixed well ,then the mixture was loaded into the wells in agarose gel. Electric current was applied, 80 volts for 1hour. Finally, the bands were visualized at wave length 350 nm on a UV transiluminator apparatus.

3.2.5.2.3: Preparation of Primers Solution:-

The primers stock solution were prepared in a final concentration (pmol/ µl) ,as described for each primers by dissolving the lyophilized oligonucleotide in deionized water .For prepared(10 pmol) concentration of work solution ,10 µl of stock solution were re-suspended in 90 µl of deionized water.

3.2.5.2.4: Master Mix:-

The master mix components were mentioned in table (3-7) and (3-8):

Table (3-7): Master Mix (AccuPower®PCR PreMix) used in Monoplex PCR.

No.	Component	Reaction volume 25 µl reaction
1-	Taq DNA polymerase	1U
2-	dNTPs (dATP, dCTP, dGTP, dTTP)	250 µM each
3-	Tris-HCL(PH 9.0)	10 mM
4-	KCl	30 mM
5-	MgCl2	1.5 mM
6-	Sterilizer and tracking dye1	Trace

Table (3-8): Master Mix (AccuPower®Multiplex PCR PreMix) used in Multiplex PCR:

No.	Component	Amount (25µl)
1-	Taq DNA polymerase	1U
2-	Pyrophosphatase and pyrophosphate	
3-	dNTPs (dATP, dCTP, dGTP, dTTP	250 µM each
4-	Reaction Buffer with 2Mm MgCl2	1x
5-	Sterilizer and tracking dye	

3.2.5.2.5: Monoplex PCR Protocol:-

The protocol used according to the instructions of the manufacturer Bioneer . All components of PCR were assembled in PCR tube and mixed by cooling microcentrifuge for 10 seconds at 850 rpm.

The Steps of Monoplex PCR were Conducted as Following :-

1- Template DNA and Primers were dissolved before usage.

2- Primers and template DNA were added into the AccuPower®. Taq premix tubes as shown in table (3-9).

3-The lyophilized blue pellets were completely dissolved and spin down by using vortex apparatus.

4- The PCR Eppendorf tubes were placed in the thermocycler apparatus.

The appropriate PCR program conditions with some modifications according to Miyoshi *et al*., (2005) for universal primer shown in table (3-10).

Table (3-9) : The volume of mixture of PCR.

NO.	PCR Master mix	Volume (µl)
1-	DNA template	4 µl

2-	Forward primer	1 µl
3-	Reverse primer	1 µl
4-	Master Mix	5 µl
5-	Free inonized water	14 µl
6-	Final volume	25 µl

Table (3-10): Monoplex PCR program.

PCR step	Temperature(ºC)	Time	Repeat	Reference
Initial denaturation	94	1min	1	Miyoshi *et al.*(2005)
Denaturation	94	1min	30 cycle	
Annealing	52	35sec		
Extension	72	1min		
Final extension	72	7min	1	

3.2.5.2.6: Multiplex PCR Protocol:-

The protocol used according to the instructions of the manufacturer Bioneer. All components of multiplex PCR were assembled in PCR tube and mixed by cooling microcentrifuge for 10 sec at 850 rpm.

The Steps of Multiplex PCR were Conducted as Following :

1- Before used, specific primers and template DNA should be dissolved.

2-Template DNA and specific primers were added into the AccuPower®. Multiplex PCR Taq premix tubes as shown in table (3-11).

3- The lyophilized green pellets were completely dissolved and spin down by using vortex apparatus.

4- PCR (Eppendorf tube) were placed in the thermocycler apparatus .

The appropriate PCR program conditions with some modifications according to Lei *et al.*, *(*2008) . as shown in table (3-12).

Table (3-11) : The volume of mixture of Multiplex PCR.

NO.	PCR Master mix	Volume (µl)
1-	DNA template	1 µl
2-	Sex special primers	1 µl for each one
3-	Master Mix	5 µl
4-	Free inonized water	13µl
5-	Final volume	25 µl

Table (3-12): Multiplex PCR program.

PCR step	Temperature(ºC)	Time	Repeat	Reference
Initial denaturation	95	3min	1	Lei *et al* .(2008)
Denaturation	95	60sec	35 cycle	
Annealing	55	90sec		
Extension	72	90sec		
Final extension	72	10 min	1	
Hold	4			

3.3: Statistical Analysis:-

Extract the mean and standard deviations of total aerobic plate count, and the data were analyzed using the statistical program Social Package of Social Sciences (SPSS) version 22, using independent samples T-test to calculate the statistical differences (Al- Rawi and Khalf Allah, 2000).

CHAPTER FOUR

RESULTS

4. Results :-

4.1: Samples collection:

In present study , 100 samples of frozen food randomly collected from the commercial markets in Misan Governorate in a period of six months starting from November 2020 till April 2021 , the samples were immediately transferred under aseptic conditions to the laboratory .

4.2 : Isolation and Identification of Bacteria :-

4.2.1: Isolation and Numeration of Bacteria by Total Aerobic Plate Count (APC):-

Forty-one of bacterial isolates were isolated from frozen food samples (meat and poultry products) and numerated by using serial dilutions on the nutrient agar ,it is easy to perform and a large number of organisms can be counted as CFU/ml,as show in figure (4-1). Statistical analysis results of total aerobic plate count results showed in table (4-1) and table (4-2).

The results in table (4-1) show that the imported burger ,sausage and shawarma samples had mean values $2.52\times10^{13\,b}\pm1.44\times10^{13}$, $3.86\times10^{13\,b}\pm2.92\times10^{13}$and $2.78\times10^{13\,a}\pm1.12\times10^{13}$respectively while the local samples have mean values which were$2.32\times10^{13\,b}\pm1.06\times10^{13}$, $2.85\times10^{13\,b}\pm0.16\times10^{13}$ and $2.99\times10^{13\,a}\pm2.18\times10^{13}$ respectively ,so the imported samples of these three types of meat products had highest value of APC than that of local samples and the statistical analysis of results revealed that there were a significant differences ($P<0.05$) between imported and local burger and sausage samples while there were no significant differences($P>0.05$) between imported and local shawarma samples .

The minced meat samples from L1 have mean value $5.56\times10^{12\,a}\pm3.10\times10^{12}$higher than the samples of L2 which have $2.59\times10^{13\,a}\pm0.4\times10^{13}$ so the samples from L1

had highly values of APC than the L2 samples , kebab from L1 have mean value 2.46×10^{13} ^{b} $\pm1.81\times10^{13}$while the samples from L2 which have mean value 1.24×10^{13} ^{b} $\pm$ 0.19×10^{13} so the kebab samples from L1 have highly APC than the samples from L2. The statistical analysis of results for these two products showed that there were significant differences($P<0.05$) between L1 and L2 of kebab samples while there was no significant differences ($p>0.05$) between L1 and L2 of minced meat samples.

Table (4-1): Statistical analytical results of total Aerobic Plate Count (APC) in the examined samples of meat products (n=50).

Meat product	Type	Min*	Max*	Mean ± Std Dev*
Burger	I	1.72×10^{13}	3.20×10^{13}	2.52×10^{13} ^{b} $\pm1.44\times10^{13}$
	L	8.30×10^{12}	2.13×10^{13}	2.32×10^{13} ^{b} $\pm1.06\times10^{13}$
Sausage	I	5.60×10^{12}	1.92×10^{13}	3.86×10^{13} ^{b} $\pm2.92\times10^{13}$
	L	2.57×10^{13}	2.98×10^{13}	2.85×10^{13} ^{b} $\pm0.16\times10^{13}$
Minced meat	L1	1.12×10^{13}	8.3×10^{12}	5.56×10^{12} ^{a}$\pm3.10\times10^{12}$
	L2	2.32×10^{13}	3.3×10^{13}	2.59×10^{13} ^{a}$\pm0.4\times10^{13}$
Shawarma	I	1.28×10^{13}	4.1×10^{12}	2.78×10^{13} ^{a}$\pm1.12\times10^{13}$
	L	1.25×10^{13}	6.6×10^{12}	2.99×10^{13} ^{a}$\pm2.18\times10^{13}$
Kebab	L1	1.87×10^{13}	2.91×10^{13}	2.46×10^{13} ^{b} $\pm1.81\times10^{13}$
	L2	3.10×10^{12}	2.67×10^{13}	1.24×10^{13} ^{b} $\pm$ 0.19×10^{13}

Note: Std Dev* = standard deviation of mean / Min* = minimum / Max*= maximum/ I= imported / L= local. The value represents (mean± Std Dev), Vertically similar letters indicate that there are no significant differences ($P>0.05$), different letters vertically between the values indicate that there are significant differences ($P<0.05$).

The results in table (4-2) showed that the imported liver ,chest ,thighs and burger samples had mean values which were 1.89×10^{13} ^{b} $\pm1.22\times10^{13}$, 2.91×10^{13} ^{b} $\pm2.16\times10^{13}$, 2.78×10^{13} ^{b} $\pm1.26\times10^{13}$ and 2.59×10^{13} ^{b} $\pm2.07\times10^{13}$ respectively while

the local samples of these type have mean value 9.2×10^{12} ᵇ $\pm4.7\times10^{12}$, 1.83×10^{13} ᵇ $\pm1.19\times10^{13}$, 1.64×10^{13} ᵇ $\pm0.13\times10^{13}$ and 1.66×10^{13} ᵇ $\pm1.29\times10^{13}$ respectively, so the imported samples of these four types of poultry products had highest value of APC than that of local samples. The statistical analysis of results revealed that there were a significant differences ($P<0.05$) between imported and local samples for each type of them. The Kebab samples from L1 have mean value 2.09×10^{13} ᵃ $\pm1.23\times10^{13}$ while the samples from L2 have mean value2.23×10^{13} ᵃ $\pm1.49\times10^{13}$ so the APC of kebab samples from L2 is more than the APC of L1 samples and the statistical analysis of results for this product showed that there was no significant differences($P>0.05$) between kebab samples from L1 and L2.

Table (4-2): Statistical analysis of results of total Aerobic Plate Count (APC) in the examined samples of poultry cuts and products (n=50).

Poultry cuts products &	type	Min	Max	Mean ± Std Dev*
Liver	I	6.90×10^{12}	2.06×10^{13}	1.89×10^{13} ᵇ $\pm1.22\times10^{13}$
	L	8.80×10^{12}	9.50×10^{12}	9.2×10^{12} ᵇ $\pm4.7\times10^{12}$
Chest	I	2.45×10^{13}	3.73×10^{13}	2.91×10^{13} ᵇ $\pm2.16\times10^{13}$
	L	7.80×10^{12}	2.56×10^{13}	1.83×10^{13} ᵇ $\pm1.19\times10^{13}$
Thighs	I	2.54×10^{13}	3.00×10^{13}	2.78×10^{13} ᵇ $\pm1.26\times10^{13}$
	L	7.30×10^{12}	2.93×10^{13}	1.64×10^{13} ᵇ $\pm0.13\times10^{13}$
Kebab	L1	8.50×10^{12}	2.99×10^{13}	2.09×10^{13} ᵃ $\pm1.23\times10^{13}$
	L2	1.97×10^{13}	2.81×10^{13}	2.23×10^{13} ᵃ $\pm1.49\times10^{13}$
Burger	I	1.98×10^{13}	3.00×10^{13}	2.59×10^{13} ᵇ $\pm2.07\times10^{13}$
	L	1.55×10^{13}	2.01×10^{13}	1.66×10^{13} ᵇ $\pm1.29\times10^{13}$

Note: Std Dev* = standard deviation of mean / Min* = minimum / Max*= maximum/ I= imported / L= local. The value represents (mean± Std Dev), Vertically similar letters indicate that there are no significant differences ($P>0.05$),different letters vertically between the values indicate that there are significant differences ($P<0.05$).

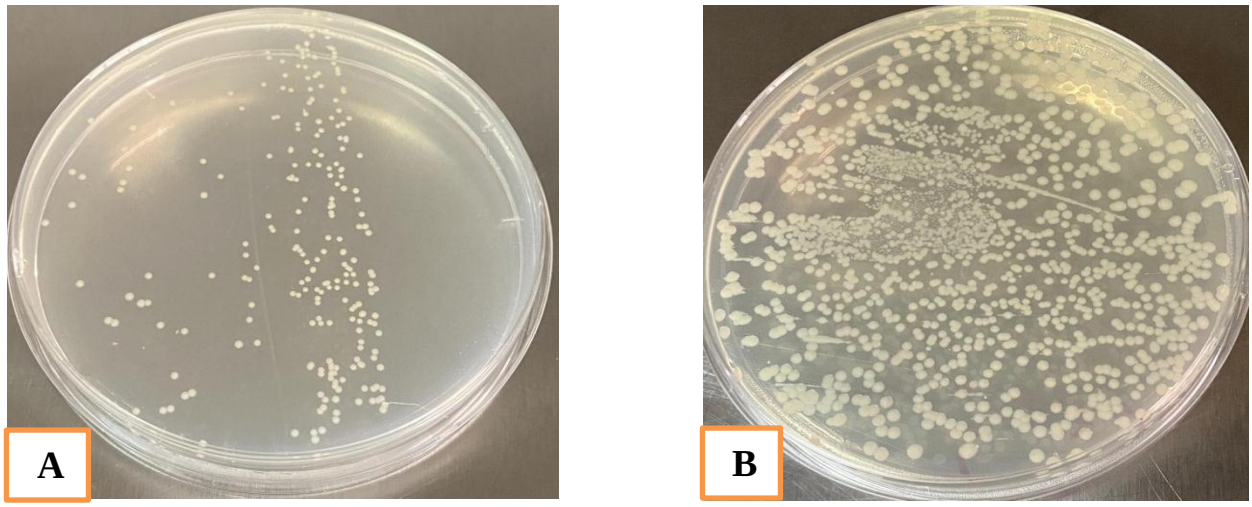


Figure (4-1):(A)The number of bacterial colonies (215), (B) the number of bacterial colonies more than 300(TNTC).

4.2.2: Preliminary Identification of Bacteria:-

Several subcultures were done to obtain pure culture of bacteria (Appendix1). Gram stain was showed that Gram negative bacteria was most common in meat product (52%) while Gram positive bacteria represent (48%) . In poultry samples Gram stain showed that Gram positive bacteria was the most dominant isolates (59%) with (41%) the percentage of Gram negative bacteria .

The forms of bacteria were ranged from cocci, bacilli and variable pleomorphic (Appendix 2).

All the bacterial isolates have shown good growth on the blood agar with different patterns of hemolysis (Appandix 3A). The Gram positive bacteria didn't show any growth on the MacConkey agar while the Gram negative bacteria were grow very well (Appandix 3B).

4.2.3: Diagnosis of Bacteria by Vitek2 Compact System :-

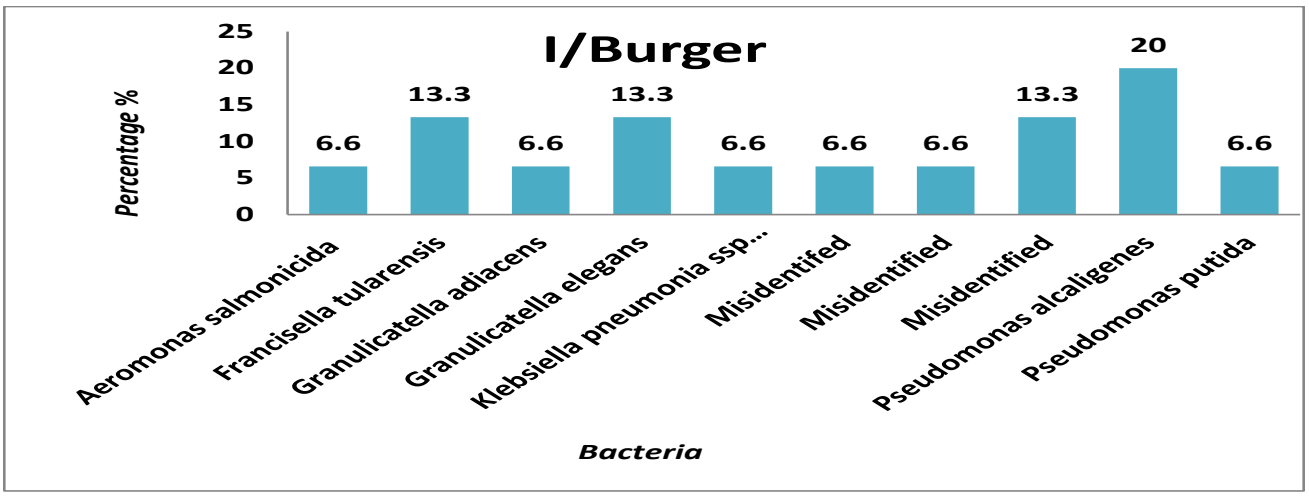
The characteristic of bacterial growth on selective media is not enough for final detection of bacteria. All isolates should be examine biochemically, in this study we used Vitek2 compact system for this purpose ,only five bacterial isolates were not detected by this system .The result in table (4-3),Appendix (4) and in figures (4-2),(4-3) showed the type and percentage of bacteria that isolated from meats and poultry products samples .

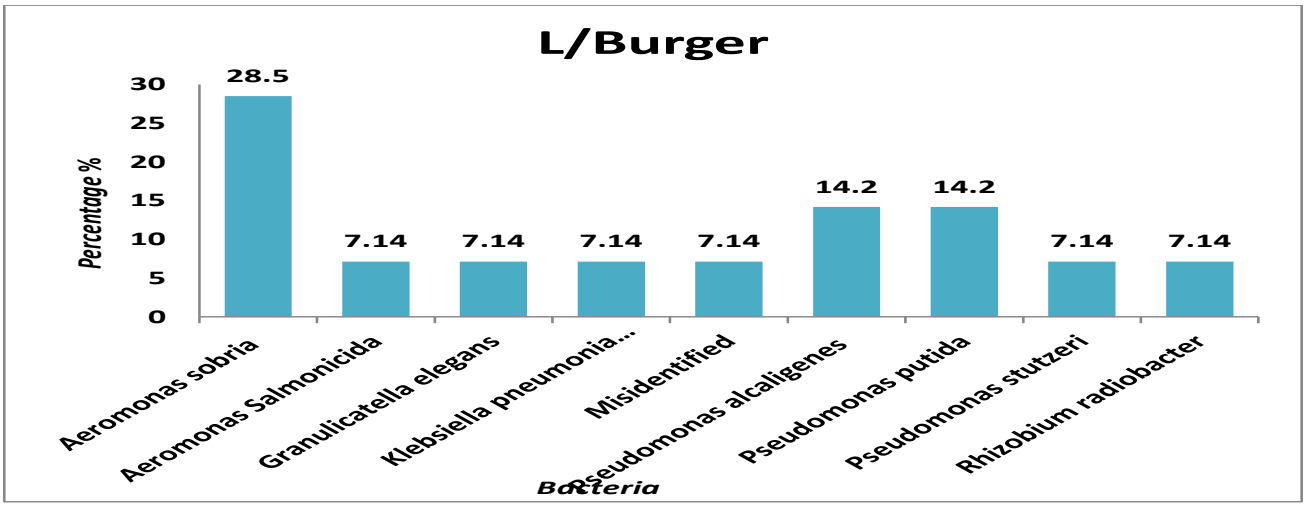
Table (4-3) : The results of vitek2compact system to bacteria that isolated from meat and poultry products samples .

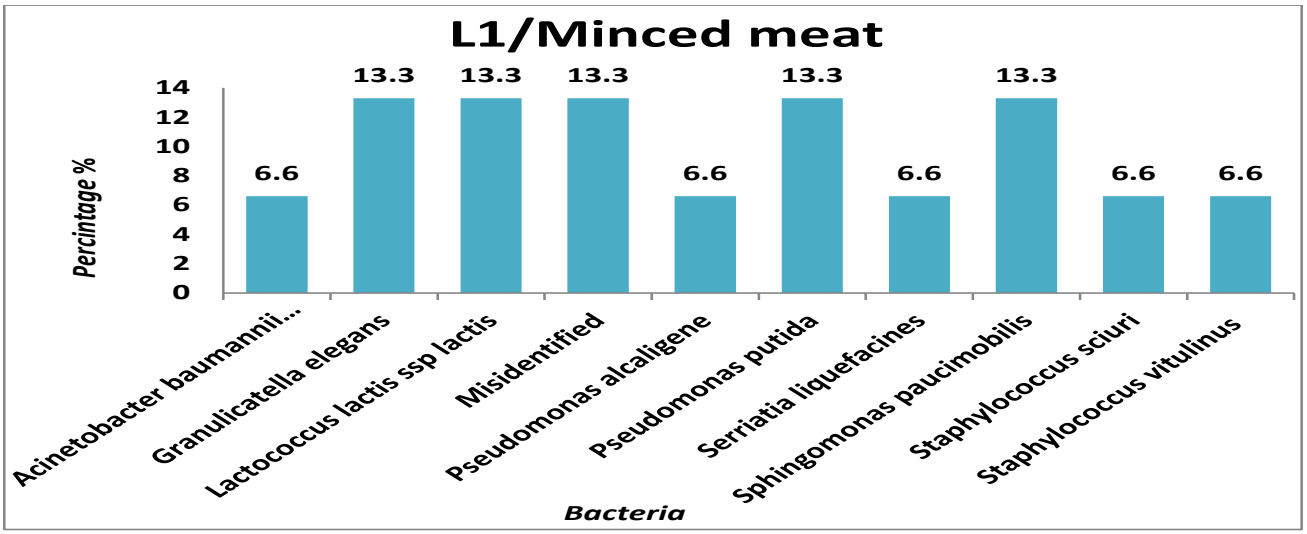
Bacterial Isolate	Bacterial identification by vitek2 compact
M1	*Pseudomonas alcaligenes*
M2	*Aeromonas salmonicida*
M3	*Klebsiella pneumonia* ssp. pneumonia
M4	*Francisella tularensis*
M5	*Pseudomonas stutzeri*
M6	*Aeromonas sobria*
M7	*Acinetobacter baumannii* complex
M8	*Pseudomonas putida*
M9	*Serratia liquefaciens* group
M10	*Pseudomonas oryzihabitans*
M11	Misidentified
M12	*Granulicatella elegans*
M13	*Granulicatella adiacens*
M14	*Staphylococcus sciuri*
M15	*Lactococcus lactis* ssp. lactis
M16	*Kocuria rosea*
M17	*Aerococcus viridans*
M18	Misidentified
M19	*Kocuria rhizophila*
M20	*Staphylococcus warneri*

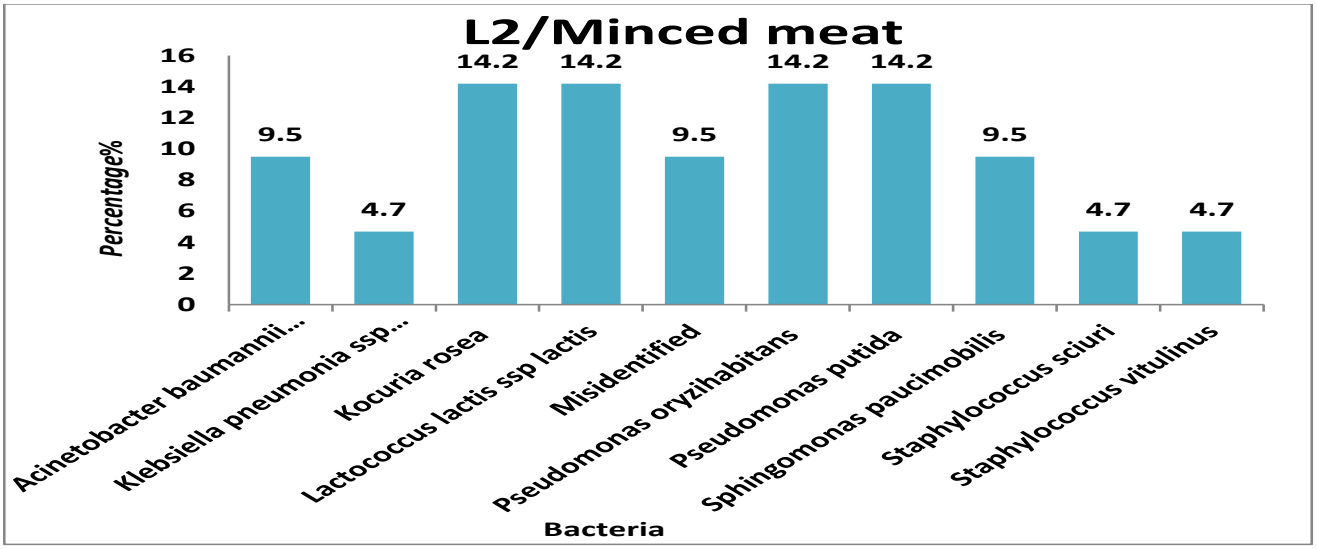
M21	***Staphylococcus epidermidis***
M22	***Staphylococus equorum***
M23	***Acintobacter lwoffii***
M24	***Enterobacter cloacae* complex**
M25	***Aerococcus urinae***
P1	***Staphylococcus lentus***
P2	***Serratia ficaria***
P3	**Misidentified**
P4	***Enterococcus columbae***
P5	***Serratia plymnthica***
P6	***Staphylococcus hominis* ssp. hominis**
P7	***Escherichia coli***
P8	***Staphylococcus auricularis***
P9	***Sphingobacterium spiritivorum***
P10	***Rosomonas gilardii***
P11	***Klebsiella oxytoca***
P12	***Sphingomonas paucimobilis***
P13	***Staphylococcus vitulinus***
P14	***Rhizobium radiobacter***
P15	**Misidentified**
P16	**Misidentified**

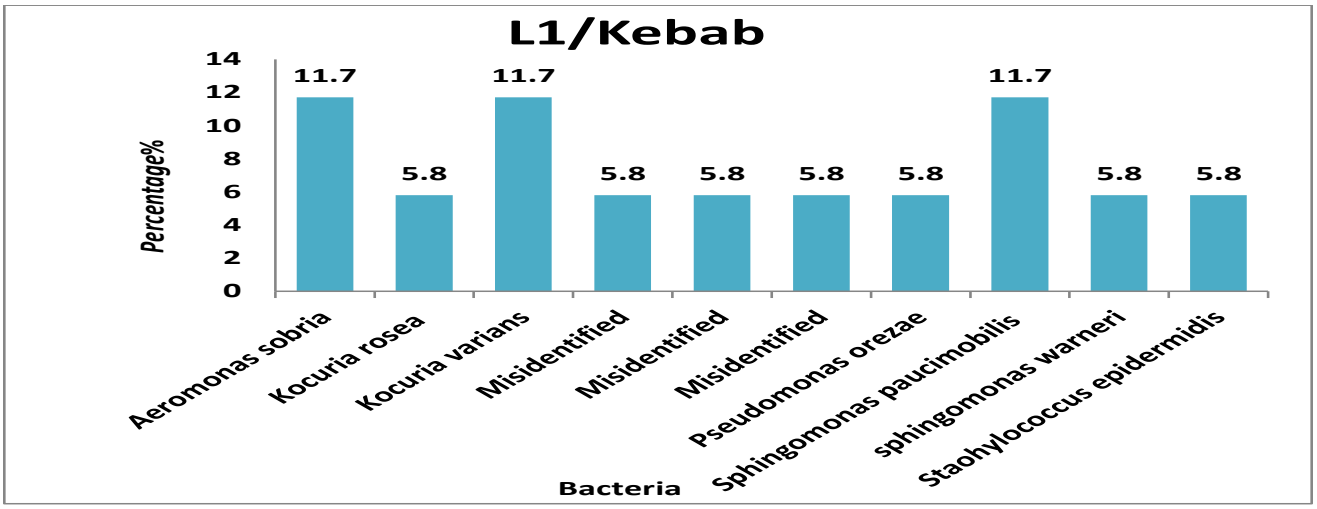
Note: M= Meat samples / P = Poultry samples .

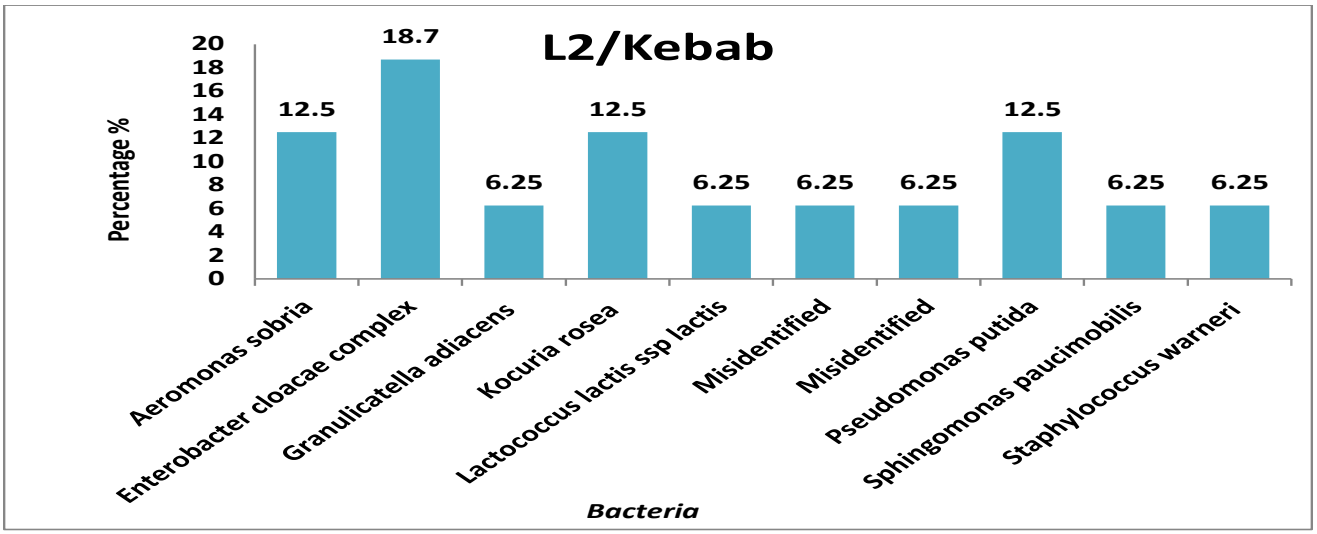


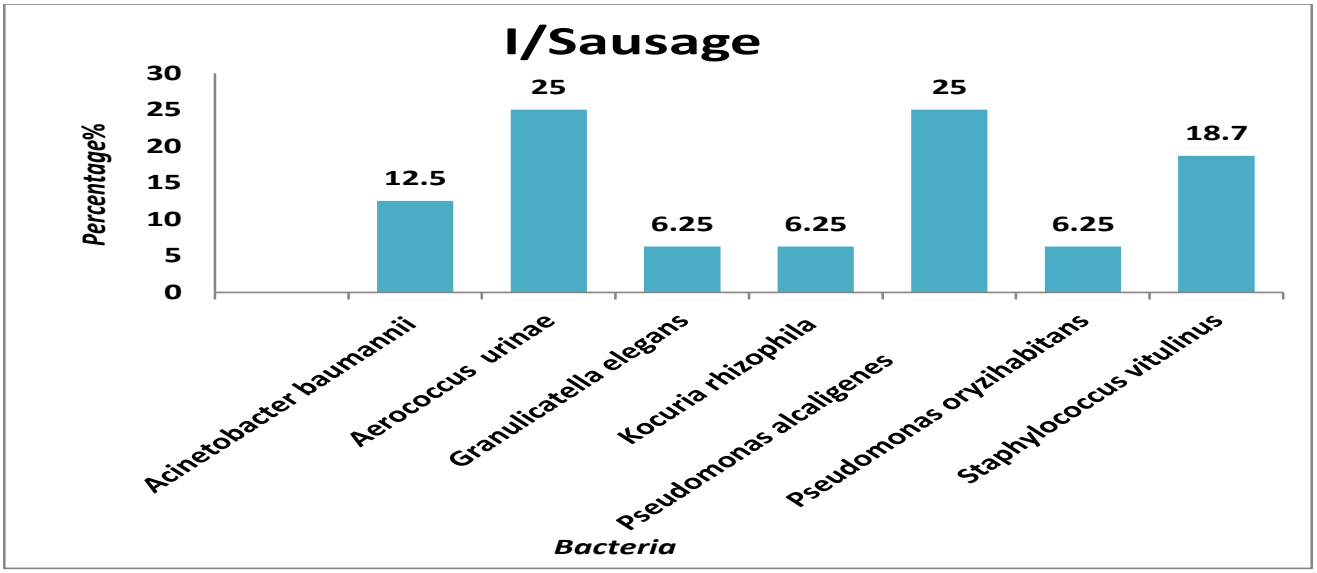

L/Burger
Percentage %
30
25
20
15
10
5
0
28.5
7.14
7.14
7.14
7.14
14.2
14.2
7.14
7.14
Aeromonas sobria
Aeromonas Salmonicida
Granulicatella elegans
Klebsiella pneumonia...
Misidentified
Pseudomonas alcaligenes
Pseudomonas putida
Pseudomonas stutzeri
Rhizobium radiobacter
Bacteria

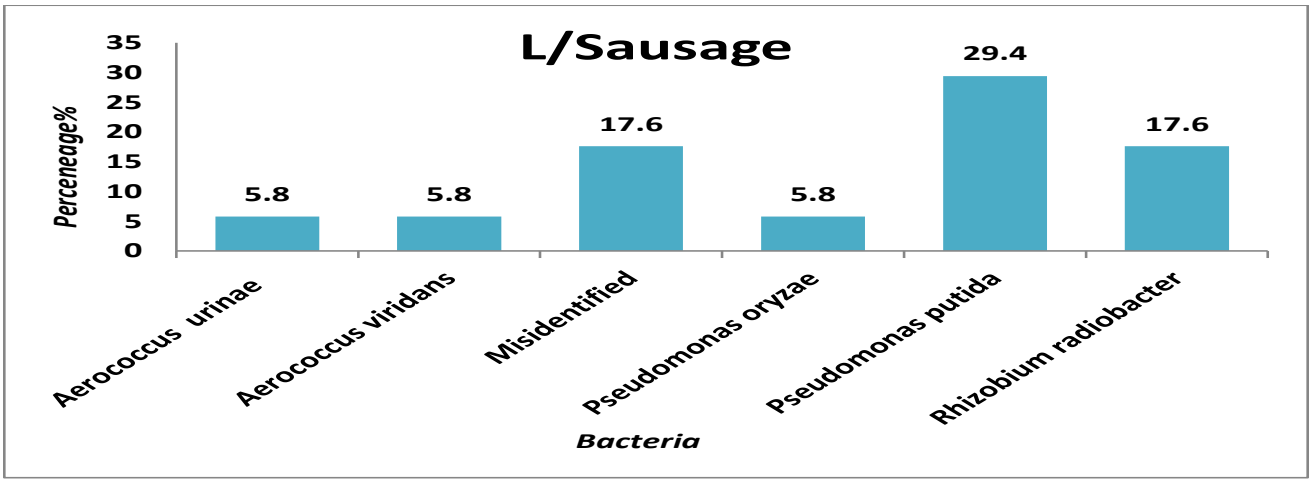

L1/Minced meat
Percintage %
14
12
10
8
6
4
2
0
6.6
13.3
13.3
13.3
6.6
13.3
6.6
13.3
6.6
6.6
Acinetobacter baumannii...
Granulicatella elegans
Lactococcus lactis ssp lactis
Misidentified
Pseudomonas alcaligene
Pseudomonas putida
Serriatia liquefacines
Sphingomonas paucimobilis
Staphylococcus sciuri
Staphylococcus vitulinus
Bacteria

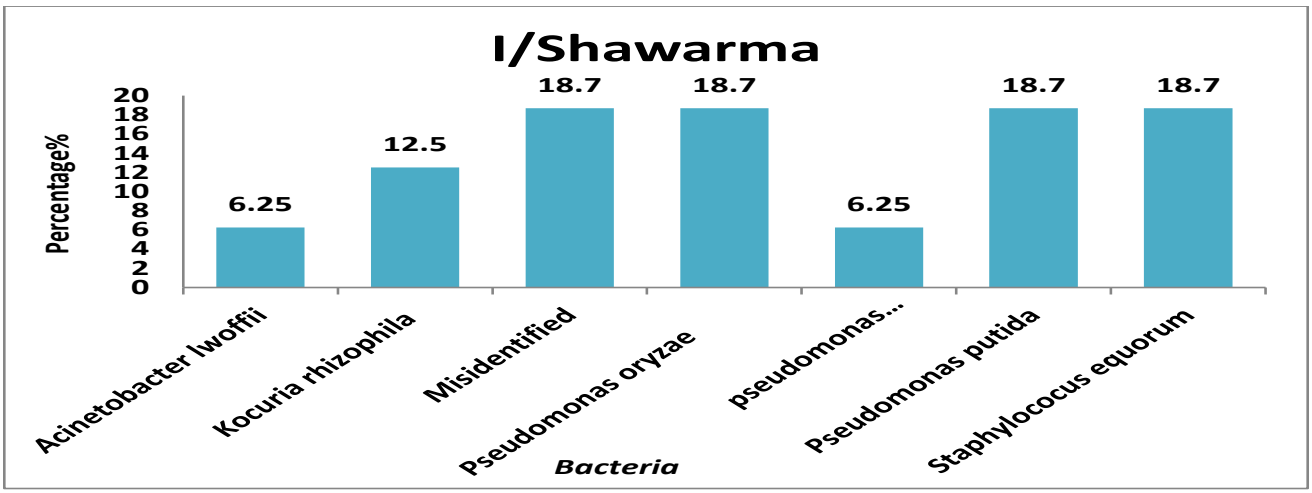

L2/Minced meat
Percentage%
16
14
12
10
8
6
4
2
0
9.5
4.7
14.2
14.2
9.5
14.2
14.2
9.5
4.7
4.7
Acinetobacter baumannii...
Klebsiella pneumonia ssp...
Kocuria rosea
Lactococcus lactis ssp lactis
Misidentified
Pseudomonas oryzihabitans
Pseudomonas putida
Sphingomonas paucimobilis
Staphylococcus sciuri
Staphylococcus vitulinus
Bacteria

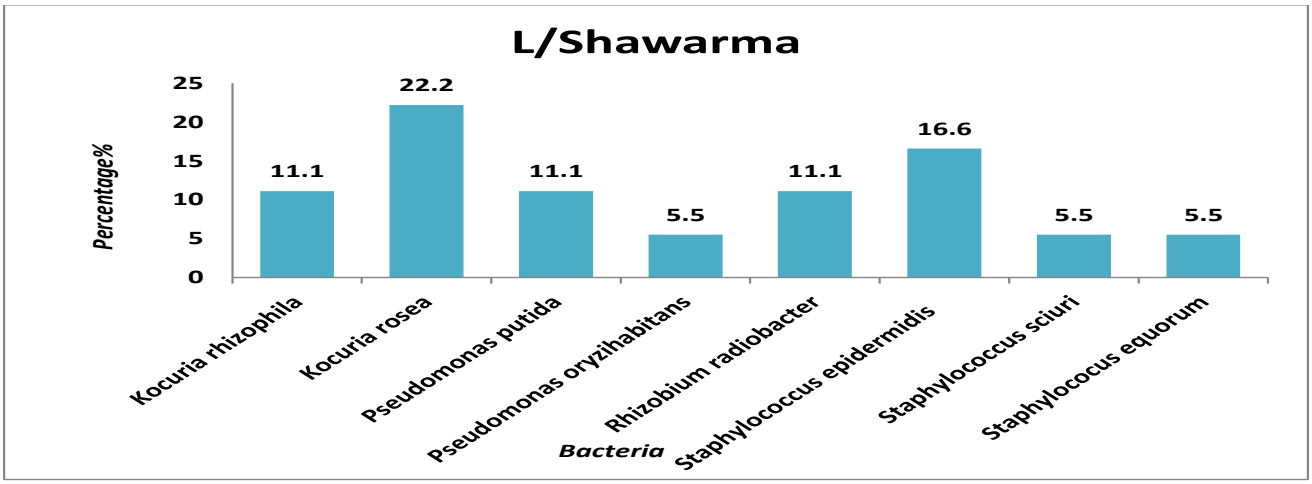

L1/Kebab
Percentage%
14
12
10
8
6
4
2
0
11.7
5.8
11.7
5.8
5.8
5.8
5.8
11.7
5.8
5.8
Aeromonas sobria
Kocuria rosea
Kocuria varians
Misidentified
Misidentified
Misidentified
Pseudomonas orezae
Sphingomonas paucimobilis
sphingomonas warneri
Staohylococcus epidermidis
Bacteria


L2/Kebab
Percentage %
20
18
16
14
12
10
8
6
4
2
0
12.5
18.7
6.25
12.5
6.25
6.25
6.25
12.5
6.25
6.25
Aeromonas sobria
Enterobacter cloacae complex
Granulicatella adiacens
Kocuria rosea
Lactococcus lactis ssp lactis
Misidentified
Misidentified
Pseudomonas putida
Sphingomonas paucimobilis
Staphylococcus warneri
Bacteria


I/Sausage
Percentage%
30
25
20
15
10
5
0
12.5
25
6.25
6.25
25
6.25
18.7
Acinetobacter baumannii
Aerococcus urinae
Granulicatella elegans
Kocuria rhizophila
Pseudomonas alcaligenes
Pseudomonas oryzihabitans
Staphylococcus vitulinus
Bacteria



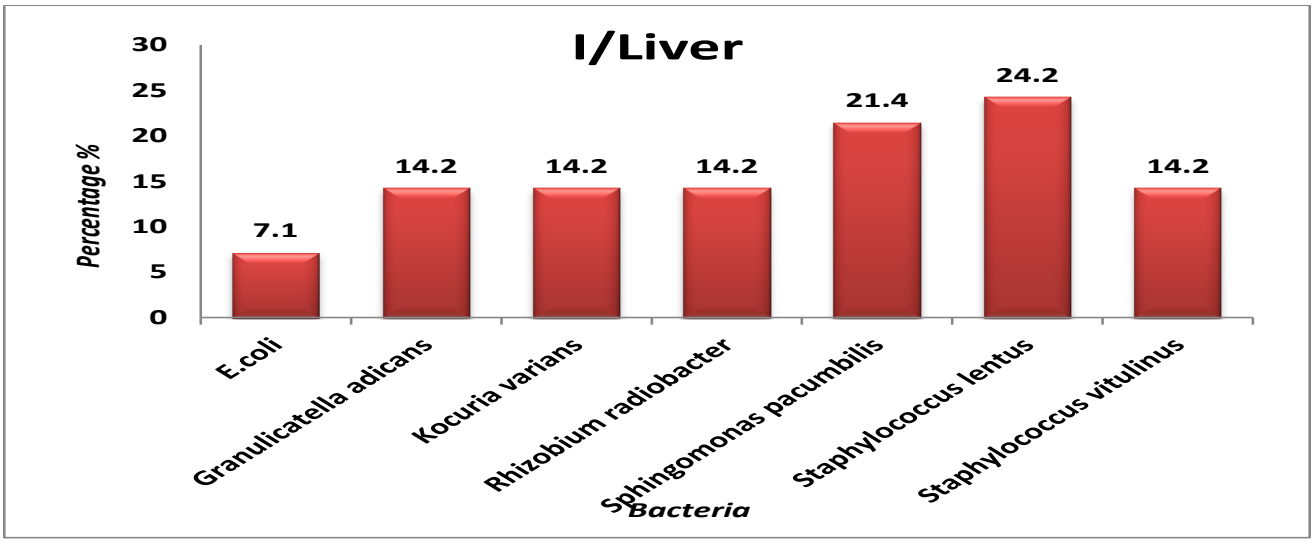


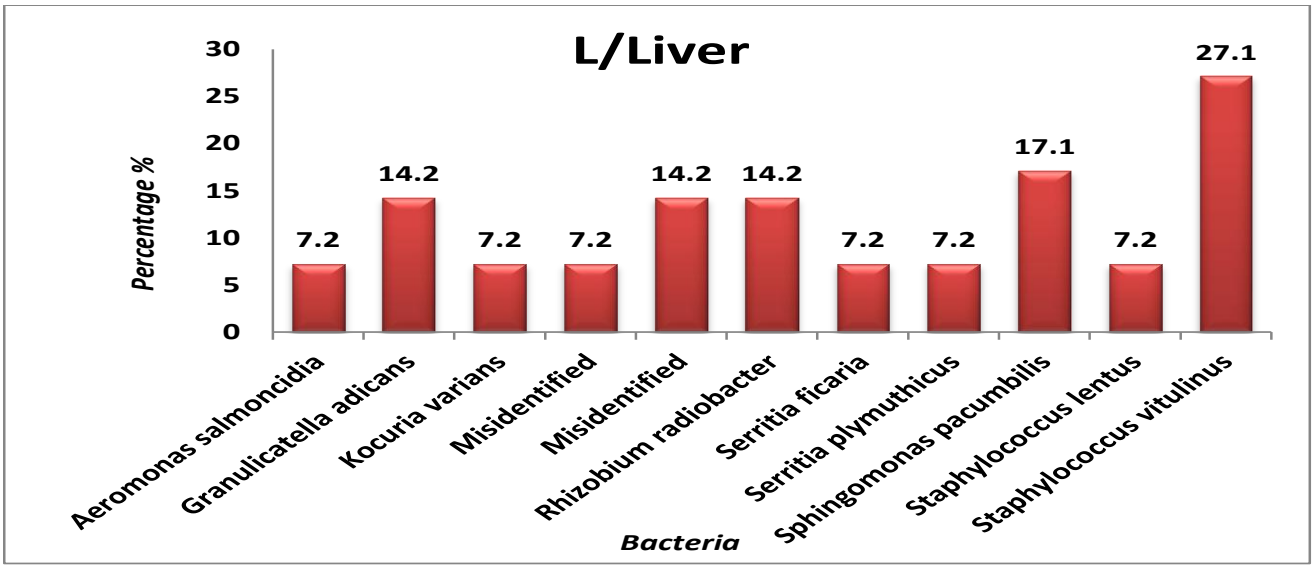


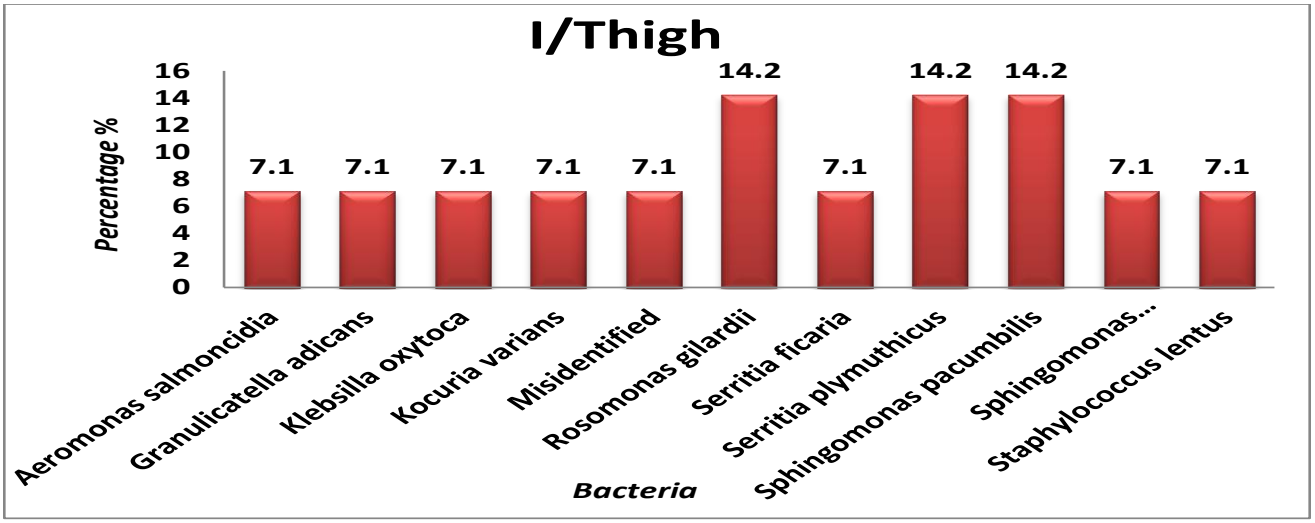
Figures (4-2): The percentage of bacteria that isolated from meat products samples

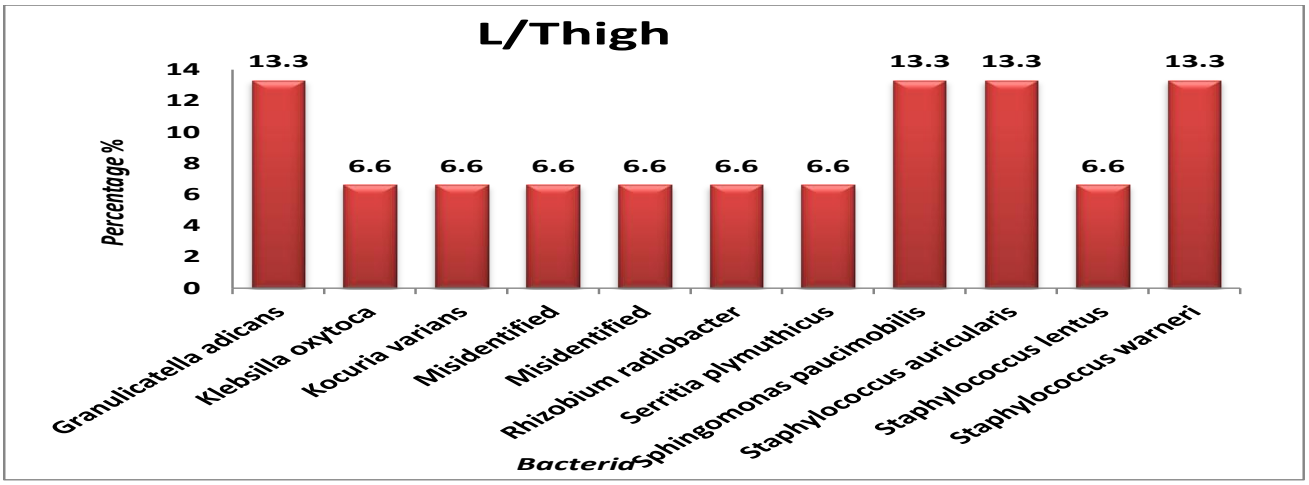
.

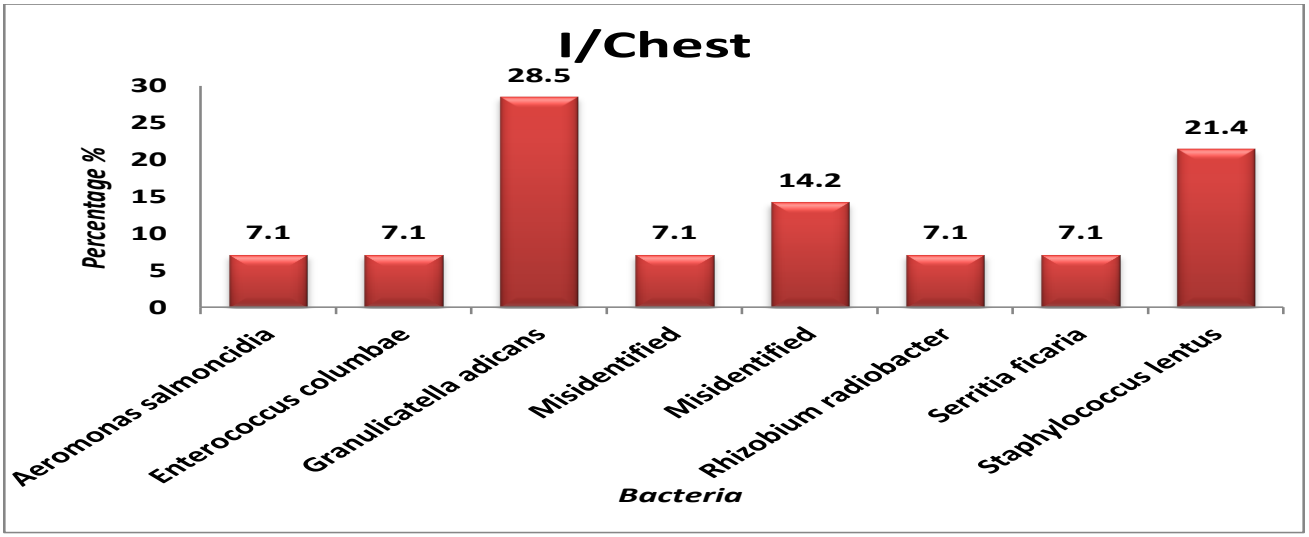
Note: I= Imported samples L= Local samples

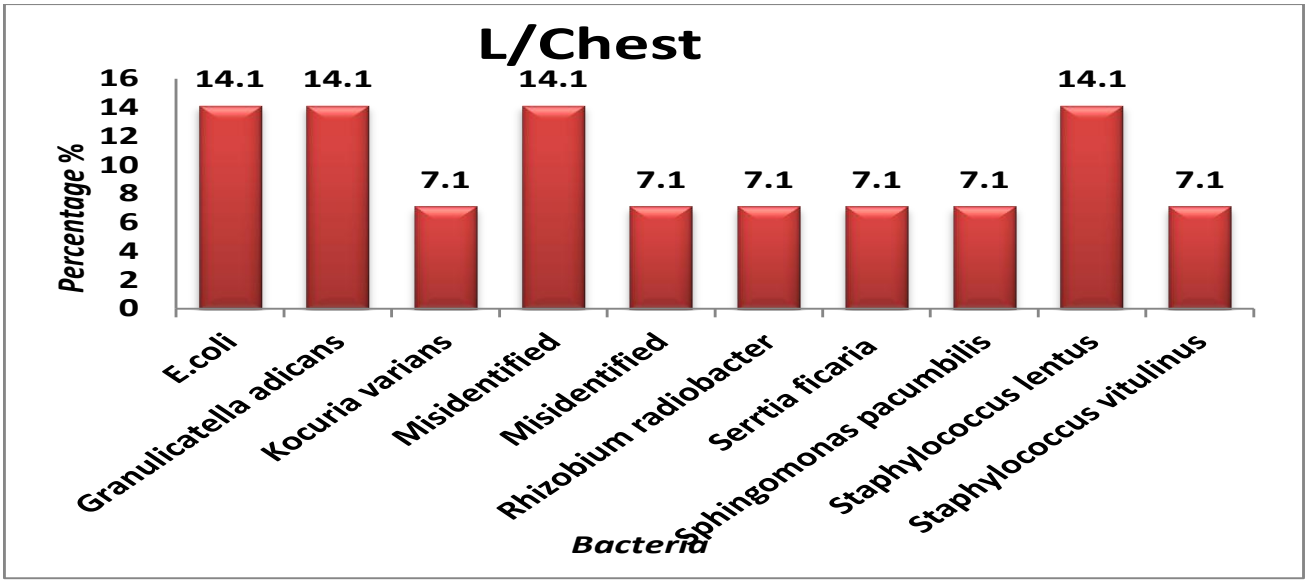

I/Liver
Percentage %
30
25
20
15
10
5
0
7.1
14.2
14.2
14.2
21.4
24.2
14.2
E.coli
Granulicatella adicans
Kocuria varians
Rhizobium radiobacter
Sphingomonas pacumbilis
Staphylococcus lentus
Staphylococcus vitulinus
Bacteria

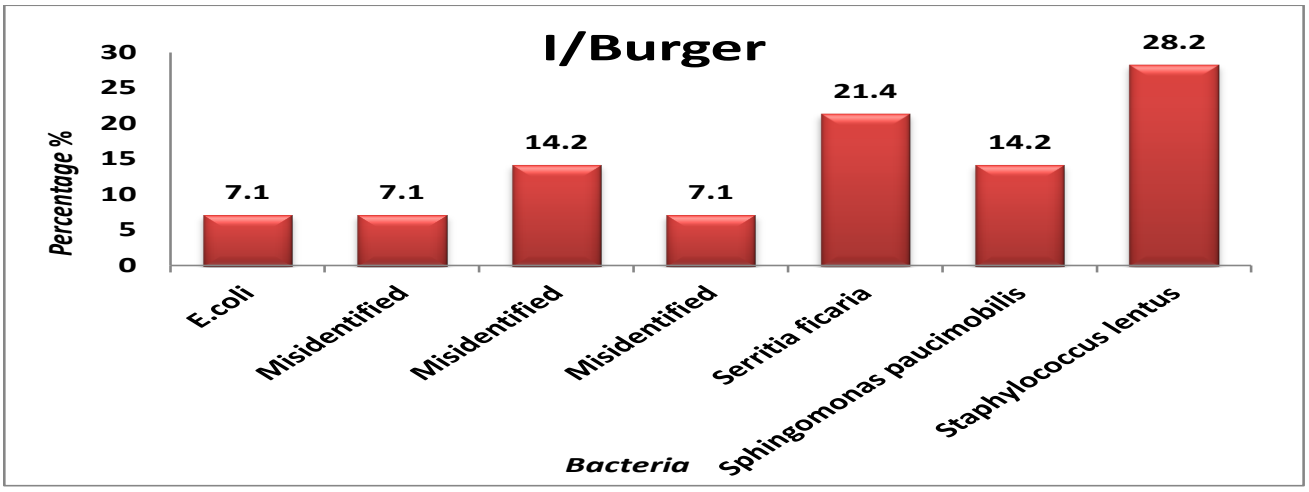

L/Liver
Percentage %
30
25
20
15
10
5
0
7.2
14.2
7.2
7.2
14.2
14.2
7.2
7.2
17.1
7.2
27.1
Aeromonas salmoncidia
Granulicatella adicans
Kocuria varians
Misidentified
Misidentified
Rhizobium radiobacter
Serritia ficaria
Serritia plymuthicus
Sphingomonas pacumbilis
Staphylococcus lentus
Staphylococcus vitulinus
Bacteria

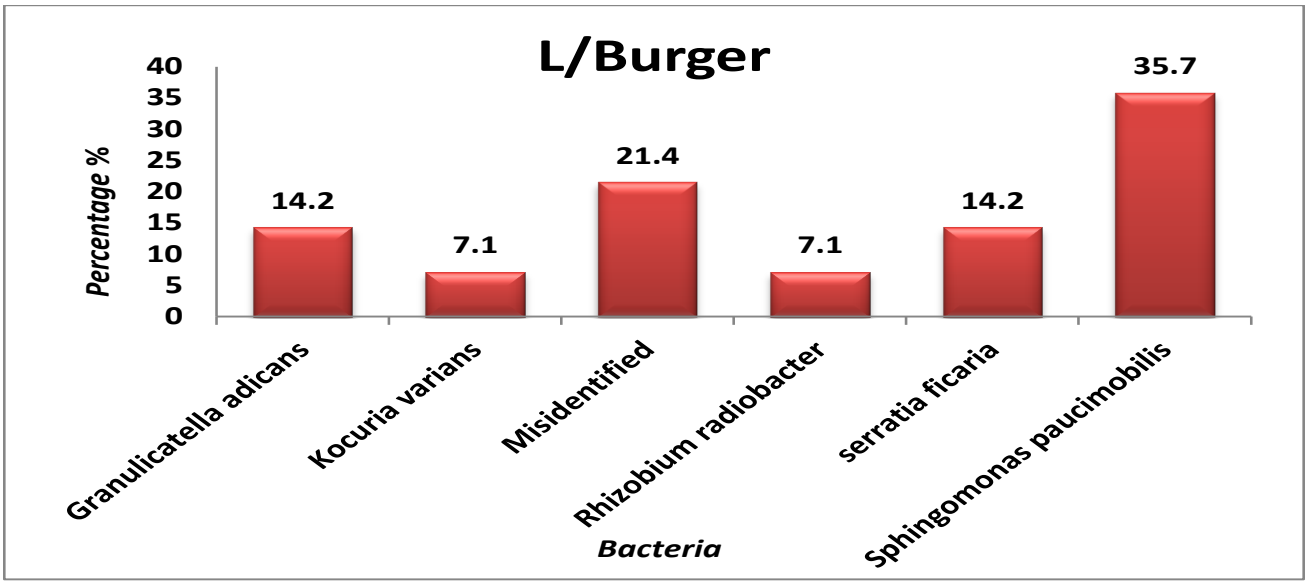

I/Thigh
Percentage %
16
14
12
10
8
6
4
2
0
7.1
7.1
7.1
7.1
7.1
14.2
7.1
14.2
14.2
7.1
7.1
Aeromonas salmoncidia
Granulicatella adicans
Klebsilla oxytoca
Kocuria varians
Misidentified
Rosomonas gilardii
Serritia ficaria
Serritia plymuthicus
Sphingomonas pacumbilis
Sphingomonas...
Staphylococcus lentus
Bacteria

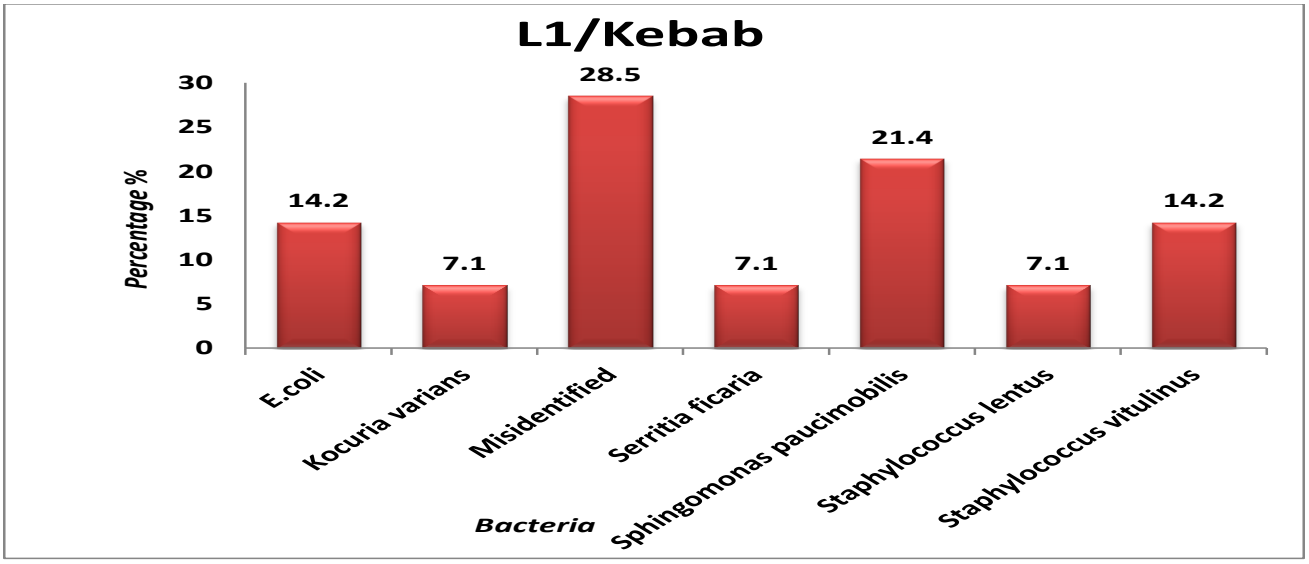

L/Thigh
Percentage %
14
12
10
8
6
4
2
0
13.3
6.6
6.6
6.6
6.6
6.6
6.6
13.3
13.3
6.6
13.3
Granulicatella adicans
Klebsilla oxytoca
Kocuria varians
Misidentified
Misidentified
Rhizobium radiobacter
Serritia plymuthicus
Sphingomonas paucimobilis
Staphylococcus auricularis
Staphylococcus lentus
Staphylococcus warneri
Bacteria

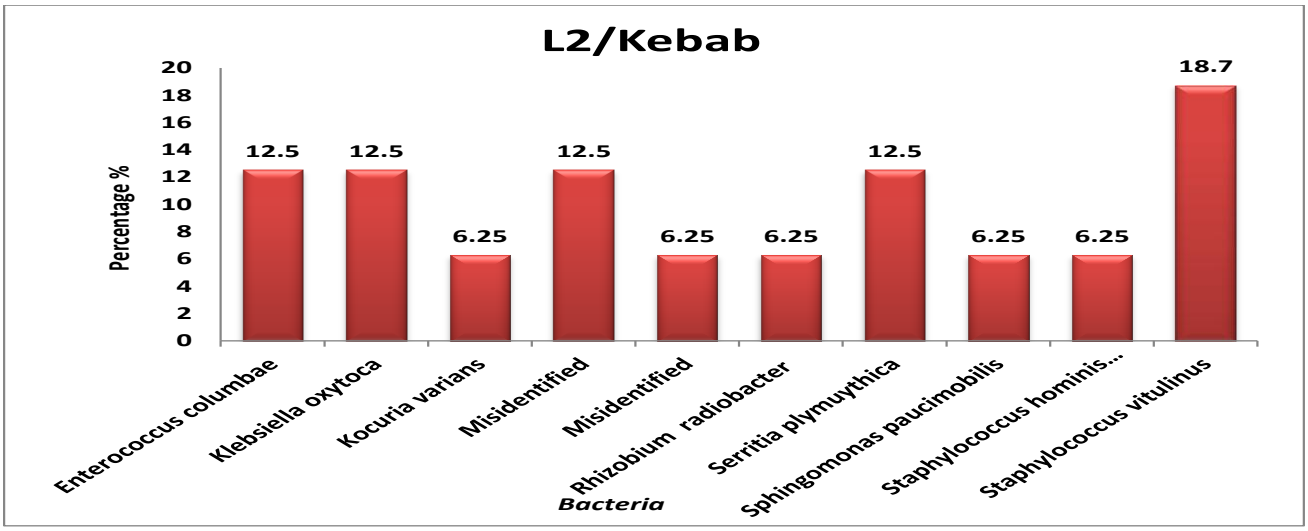

I/Chest
Percentage %
30
25
20
15
10
5
0
7.1
7.1
28.5
7.1
14.2
7.1
7.1
21.4
Aeromonas salmoncidia
Enterococcus columbae
Granulicatella adicans
Misidentified
Misidentified
Rhizobium radiobacter
Serritia ficaria
Staphylococcus lentus
Bacteria


L/Chest
Percentage %
16
14
12
10
8
6
4
2
0
14.1
14.1
7.1
14.1
7.1
7.1
7.1
7.1
14.1
7.1
E.coli
Granulicatella adicans
Kocuria varians
Misidentified
Misidentified
Rhizobium radiobacter
Serrtia ficaria
Sphingomonas pacumbilis
Staphylococcus lentus
Staphylococcus vitulinus
Bacteria


I/Burger
Percentage %
30
25
20
15
10
5
0
7.1
7.1
14.2
7.1
21.4
14.2
28.2
E.coli
Misidentified
Misidentified
Misidentified
Serritia ficaria
Sphingomonas paucimobilis
Staphylococcus lentus
Bacteria


L/Burger
Percentage %
40
35
30
25
20
15
10
5
0
14.2
7.1
21.4
7.1
14.2
35.7
Granulicatella adicans
Kocuria varians
Misidentified
Rhizobium radiobacter
serratia ficaria
Sphingomonas paucimobilis
Bacteria


L1/Kebab
Percentage %
30
25
20
15
10
5
0
14.2
7.1
28.5
7.1
21.4
7.1
14.2
E.coli
Kocuria varians
Misidentified
Serritia ficaria
Sphingomonas paucimobilis
Staphylococcus lentus
Staphylococcus vitulinus
Bacteria



Figures(4-3): The percentage of bacteria that isolated from poultry products samples.

Note: I= Imported samples L= Local samples

4.2.4: Detection of Bacteria by Molecular Techniques:-

A large number of methods have been developed to detect bacteria that cause food contamination and human diseases . Among them molecular methods have become popular and reliable techniques for differentiating pathogenic strains and hygiene screening of food samples.

4.2.4.1: Multiplex Polymerase Chain Reaction (m-PCR):-

In the current study, the Multiplex PCR technique was used to ascertain the presence of common bacterial species in meat by amplification of six special primers for bacteria which did not appear in the preliminary diagnosis of bacteria, also did not appear in the results of the Vitek2compact system . No positive result was obtained, as shown in Figure (4-4).

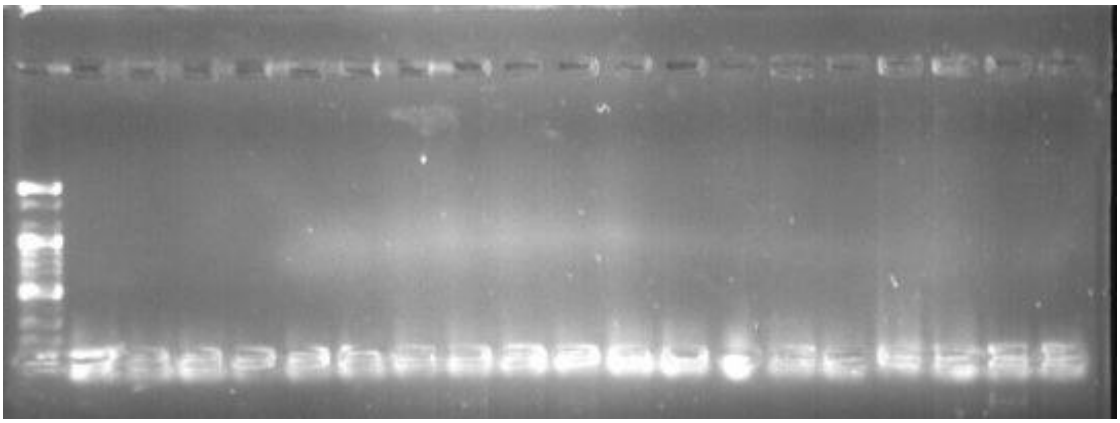


Figure (4-4):The negative results of Multiplex PCR.

4.2.4.2: Monoplex Polymerase Chain Reaction (PCR) of 16S r RNA gene :-

The bacterial universal primer pair specific to 16SrRNA gene fragment was used to identify bacterial isolates; positive results were recorded for all bacterial selected with an amplification band corresponding to 1500 bp, confirming that all isolates were bacterial species(Figure 4-5).

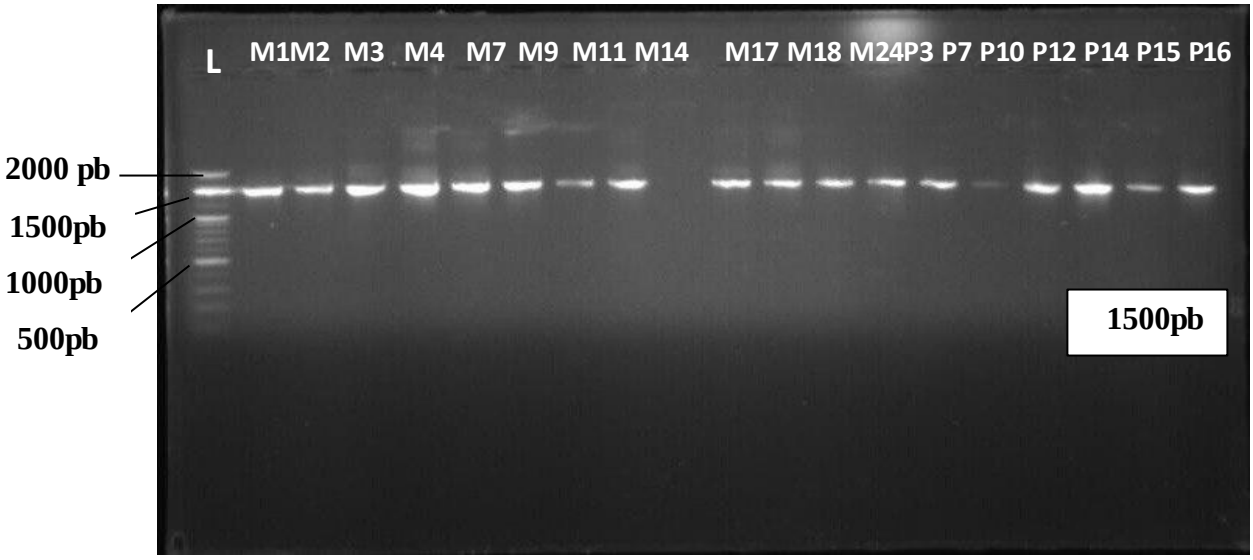


Figure (4-5) : Ethidium bromide stained gel electrophoresis of the 16s rRNA gene of bacterial isolates (1500) lane (L) represents the molecular ladder (100bp) and lanes (M1-4,7,9,11,14,17,18,24)(P3,7,10,12,14-16) represents positive PCR results on 2%agarose gel electrophoresis with 80 voltes for one hour.

From forty –one bacterial species that were identified phenotypically and confirmed by Vitek2 compact system, eighteen bacterial species were selected for the homology studies by used 16S r RNA sequence and Pairwise Sequence Alignment of the Sequencing data were obtained by using NCBI-BLAST(Nucleotide BLAST), the result represented in table (4-4), (Appendix 5).

Table(4-4) : Bacterial identification based on 16S rRNA sequencing data.

Bacterial strain	Maximum score	Total score	Query coverage	E. value	Identity Percentage	Accession No.
Acinetobacter lwoffii	1690	1690	100%	0.0	98.44%	NR-113346.1
Acinetobacter lwoffii	1711	1711	99%	0.0	97.79%	NR-026209.1
Aeromonas veronii bv.veroni	721	721	90%	0.0	83.65%	NR-119045.1
Aeromonas veronii bv.veronii	1895	1895	97%	0.0	97%	NR-119045.1
Bacillus licheniformis	1622	1622	100%	0.0	99.66%	NR-165685.1
Comamonas testosteron	65.8	131	4%	7e-10	100%	NR-029161.2
Enterobacter hormaechei **sub sp. xiangfangensis**	1439	1439	99%	0.0	96.25%	NR-126208.1
Klebsiella pneumoniae	1862	1862	97%	0.0	99.42%	NR-117683.1
Klebsiella pneumoniae	767	767	92%	0.0	95.62%	NR-113702.1
Klebsiella pneumoniae **subsp.ozaenae**	1055	1055	100%	0.0	98.82%	NR-041750.1
Lysinibacillus boronitolerans	494	494	92%	2e-139	94.14%	NR-114207.1
Methylogaea oryzae	425	425	85%	1e-118	80.67%	NR-116407.1
Pseudomonas japonica	625	625	43%	1e-178	99.71%	NR-114192.1
Pseudomonas putida	2098	2098	91%	0.0	96.54%	NR-114794.1
Pseudomonas plecoglossicida	857	857	98%	0.0	97.98%	NR-114226.1

Pseudomonas songnenensis	1711	1711	97%	0.0	95.94%	NR-148295.1
Psychrobacter sanguinis	1140	1140	97%	0.0	95.3%	NR-117833.1
Serratia liquefaciens	2156	2156	97%	0.0	95.82%	NR-121703.1

The current study was able to recorded sixteen bacterial strains in Gen Bank data belong different accession numbers as shown in table (4-6).

Table (4-5) : Recording of bacterial strains in Gen Bank.

Bacterial strain	Title in Gen Bank	Accession number
Acinetobacter lwofii	***Acinetobacter lwofii*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ931306.1
Acinetobacter lwofii	***Acinetobacter lwofii*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ921931.1
Aeromonas veronii	***Aeromonas veronii*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ934693.1
Aeromonas veronii	***Aeromonas veronii*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ931286.1
Bacillus licheniformis	***Bacillus licheniformis*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ919316.1
Enterobacter hormaechei	***Enterobacter hormaechei*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ919358.1
Klebsiella pneumoniae	***Klebsiella pneumoniae*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ930472.1
Klebsiella pneumoniae	***Klebsiella pneumoniae*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ923503.1
***Klebsiella pneumoniae* subsp.ozaenae**	***Klebsiella pneumoniae subsp.ozaenae*** strain ZD202116S ribosomal RNA gene, partial sequence	MZ926853.1
Lysinibacillus boronitolerans	***Lysinibacillus boronitolerans*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ920155.1

Pseudomonas japonica	***Pseudomonas japonica*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ927456.1
Pseudomonas putida	***Pseudomonas putida*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ913024.1
Pseudomonas plecoglossicida	***Pseudomonas plecoglossicida*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ934671.1
Pseudomonas songnenensis	***Pseudomonas songnenensis*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ927229.1
Psychrobacter sanguinis	***Psychrobacter sanguinis*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ923509.1
Serratia liquefaciens	***Serratia liquefaciens*** strain ZD2021 16S ribosomal RNA gene, partial sequence	MZ911849.1

The results in table (4-6) shows the similarities and differences in identification of selected bacteria between Vitek2 compact system and 16S r RNA sequences of PCR.

Table (4-6) : The similarities and differences in diagnosis of selected bacteria between Vitek2 compact system and 16S r RNA PCR.

NO.	Vitek2 system	16S r RNA PCR
M1	***Pseudomonas alcaligenes***	***Pseudomonas songnenensis***
M2	***Aeromonas salmonicidia***	***Pseudomonas japonica***
M3	***Klebsiella pneumoniae*** **sub sp. Pneumoniae**	***Klebsiella pneumonia*** **sub sp.ozaenae**
M4	***Francisella tularensis***	***Klebsiella pneumoniae***
M7	***Acinetobacter baumannii complex***	***Pseudomonas putida***
M9	***Serratia liquefaciens***	***Serratia liquefaciens strain***
M11	**Misidentified**	***Klebsiella pneumoniae***
M14	***Staphylococcus sciuri***	***Bacillus licheniformis***

M17	***Aerococcus viridans***	***Pseudomonas plecoglossicida***
M18	**Misidentified**	***Lysinibacillus boronitolerans***
M24	***Enterobacter cloacae complex***	***Enterobacter hormaechei* sub sp. xiangfangensis**
P3	**Misidentified**	***Psychrobacter sanguinis***
P7	***Escherichia coli***	***Aeromonas veronii bv.veronii***
P10	***Rosomonas gilardii***	***Aeromonas veronii bv.veronii***
P12	***Sphingomonas paucimobilis***	***Acintobacter lwoffii***
P14	***Rhizobium radiobacter***	***Acinetobacter lwoffii***
P15	**Misidentified**	***Comamonas testosterone***
P16	**Misidentified**	***Methylogaea oryzae***

CHAPTER FIVE

DISCUSSION

5.Discussion:-

5.1. Isolation and Identification of Bacteria :-

5.1.1: Isolation and numeration of bacteria by total aerobic plate count (APC):-

Meat ,both white and red ,is one of the most important elements of human food because it has been considered as highly desirable, nutritious and good quality protein rich food, meanwhile, meat is highly perishable because of they provide a nutrient required for growth and multiplication of different types of microorganism (Kalalou *et al*., 2004 and Sayed *et al*., 2021).

The Total Aerobic Plate Count (APC) reflect the bacterial contamination and declared the hygienic quality of meat products , poultry cuts and products(Younes *et al* ., 2019).

Results of the present investigation ,table (4-1) showed that all meat products samples contaminated with bacteria depend on the mean value of APC methods but the imported products are more contaminated compared with local products according to Iraqi standard specification , (2020) where the microbiological limits (cfu/ml) for frozen meat products were(1×10^7). The reason for this may be due to the long of storage period, the products exposed to thawing and freezing during the preservation process, the low quality of the meat used and the spread of bacteria in the meat during the preparation processes, also cleaning and disinfection is not be sufficient, whether for the equipment or surfaces used, as well as the workers in terms of personal hygiene (Abd El Tawab *et al*., 2015).

Hafez and his team (2019) worked on different cuts of frozen meat imported from many breeds American, Brazilian, Indian and Sudanese collected from shops at Cairo province/Egypt , where they found the mean value of APC of the examined imported frozen meat samples were $7.25x10^5 \pm 2.38\ x10^5$, $1.53x10^6 \pm 4.84\ x10^5$, $1.54\ x10^6 \pm 4.14\ x10^5$, and $2.35\ x10^6 \pm 5.59\ x10^5$ in American, Brazilian, Indian and Sudanese respectively . They attributed the cause of contamination to the presence of *staphylococci* during dressing and evisceration in the slaughter house, contamination

of butchers and equipment, also, exposure of Imported frozen meat to thawing and refreezing in markets and street vendors, yielding an abundant supply of water and form a good media for bacterial growth and multiplication.

It was found that the results of the current study are higher than the results of study of researcher Hassanein and his colleagues (2020) as they made a comparison between imported and local frozen meat, and the results of mean value of aerobic plate count in the examined chilled local meat samples were $4.1\times10^{7}\pm 0.02\times10^{7}$CFU/g. while mean value of the examined frozen imported samples were $2.9\times10^{7}\pm 0.02\times10^{7}$ CFU/g .Their results are explained that all examined samples, either local chilled or frozen are unaccepted for human consumption due to bad hygienic indication.

Abbas *et al*. (2014) worked on meat product samples (Beef burger, Luncheon, Pasterma and Sausage) in Alexandria province/Egypt, all the examined samples found to be contaminated with different types of microorganisms with the mean values of 8.20×10^{2},6.29×10^{2},$5.40\times10^{2}$and 8.28×10^{2}respectively.

Frazier and Westhaff (1978) and Mansour *et al*. (2019) reported that the presence of contaminated and pathogenic bacteria in meat is caused by animal infected by bacteria, soiled hooves and hair carry large quantities of bacteria from food, soil, barn or water, which are sources of contamination of the surface of the carcass after removing its skin, keep the meat with the intestines , the animal's stomach, and using the same utensils increases the likelihood of meat contamination, the butcher and open markets may be become another source of pollution when the health conditions are not suitable and poor sanitary procedures, slaughterhouse and people deal with animals may be become a source of bacterial contamination.

Results of current study, table (4-2) stated that all samples of meat chicken cuts and products were contaminated with bacteria ,but we noted that the imported samples more contaminated compare with local according to Iraqi standard specification , (2020) where the microbiological limits (cfu/ml) for frozen chicken cuts and products

were (1×10^7),and there was a significant differences ($p<0.05$) between them. This result may be due to contamination of poultry meat during the slaughtering, cutting and freezing process- stored for long periods and exposure to various physical factors such as heat and moisture- failure to follow health and hygiene conditions by workers in the field of manufacturing.

Hassanien and his colleagues (2016) reported that the reason of the higher APC in chicken meat was due to the contamination of carcasses during the slaughtering process, in addition to that, the broiler meat may be kept at room temperature, which allows the growth and reproduction of mesophilic microorganisms. The reason may also be due to the fact that the tools used in cutting and preparation are contaminated, as they are used daily without complete hygiene, and this may lead to the promotion of transmission contamination to following carcasses.

AL-Tamimi and AL-Khafaji (2021) made a comparison between imported frozen and local fresh poultry's meat in Diyala province/Iraq .They found the highest viable bacterial counts from frozen meat, neck 3.15± 0.13 x10 ^{5}, wing 2.49± 0.16 x10 ^{5}, thigh 2.28 ±0.15 x10 ^{5}, breast 1.51 ±0.38 x10 ^{5}, While from fresh meat, breast, 7.0± 0.10 x10^4, thigh 4.98 ± 0.44 x10^4, neck 3.94 ± 0.27 x10^4, and wing 3.16 ±0.10 x10^4. The results of their APC were lower than current study but the two studies proved that imported frozen poultry meat was more contaminated compare with local.

The result of current study in line with the results of Yar and his team (2020) which they worked on the frozen chicken parts from three countries in Ghana and they reported high contamination in the imported samples. they agree that the differences in bacteria counts for chicken meats in their study might be due to technologies used, hygienic conditions during slaughtering, processing, packaging, storage and distribution.

The contamination of imported and local poultry cuts and products with different microorganism (spoilage and pathogenic bacteria) from different regions studied by many of researchers like Khalifa and Abd -Elshaheed , (2005); Bohaychuk *et al.*, (2006) ; Haleem *et al.*, (2013); Murad *et al.*, (2014) and Kunadu, (2018).

5.1.2: Preliminary Identification of Bacteria :-

The results of the preliminary isolation showed the growth of bacterial colonies on the nutritional ,enrichment and differential medium {Nutrient, Blood, MacConkey agar}.All bacterial isolates grown on blood media ,some of them gave alpha hemolysis patterns and others showed beta hemolysis patterns. On MacConkey medium some species of bacteria were appeared in pink colonies (lactose fermented) and some were not lactose fermented which appeared in colorless colonies while the others didn't growth on it {Gram positive bacteria}.

In the present study we used special medium to isolated the common species of bacteria like {T.C.B.S}medium which didn't grow any bacteria on it ,{M.S.A}and {Sorbitol MacConkey} medium which some species of bacteria grown on them but the common species of bacteria (*Staphylococcus aureus* and *E.coli* O175:H7) didn't grown on them .

The results of Gram stain was showed that Gram negative bacteria was most dominant bacterial strains (52%) than Gram positive (48%) in meat products samples while in poultry samples the Gram positive bacteria represented (59%) and Gram negative represent (41%) . These results can be attributed to the quality of the meat and the percentage of its contamination with bacteria and the mechanism followed by bacteria in resisting low temperatures during freezing.

Sultana and her colleagues (2014) they reported that Gram negative bacteria represented 64.92% than Gram positive bacteria which represented 35.08%,where they were similar with current study concerning with frozen meat product samples.

The results of current study on frozen poultry samples disagreement with results of Elbasheir *et al.* (2019) which they worked on fresh and frozen chicken's carcasses and found Gram negative bacteria represented the predominant bacteria isolated from fresh chickens' carcasses (60.0%), compared to Gram positive bacteria (40.0%) and Gram negative bacteria represented the predominant bacteria isolated from frozen chickens' carcasses (57.8%), compared to Gram positive bacteria (42.2%).They concluded that

Chickens' carcasses are more susceptible for contamination during processing than storage.

Ahmed and Sabiel (2016) detect microbial contamination of beef meat products and they found the most Gram- Positive isolates were *Lactobacillus* spp. (10.7%) and *Kocuria kristinae* (8.8%) while the most Gram-negative isolates were *Proteus mirabilis* (6.8%) and *Klebsiella pneumoniae* (6.8%).

Oranusi and his team (2014) found coliform bacteria in all meat samples while the other species of bacteria like *Staphylococcus aureus*, *Bacillus* spp., *Salmonella*, *Flavobacterium*, *Listeria* and *Pseudomonas* in different percentage in samples of meat.They explained the high total aerobic bacteria and high coliform in meat samples to poor initial meat quality pre-freezing. Freezing only retard the growth and proliferation of contaminating organisms, it seldom destroy or killed the organism, thawing of the samples prior to analysis could equally have encouraged proliferation of the contaminant .These reason proved by many studies like Gill, (2001); National Research Council, (2003) and Zahid *et al*., (2010).

5.1.3: Diagnosis of Bacteria by Vitek2 Compact System :-

The Vitek2 compact system is developed based on fluorescence technology and they were designed for the identification of wide range of microorganisms including Gram negative and Gram positive bacteria, *Neisseriacea* and yeasts in clinical or industrial samples. There are many different marked cards containing 64 chambers for identification tests or antibiotic susceptibility testing (Darbandi, 2011).

In the present study, from forty one bacterial species only five didn't diagnosis by vitek2 compact system.

The results of current study in table (4-3) ,figure (4-2) showed the type of bacteria that isolated from meat products which diagnosed by vitek2 system .

The present study found that *Pseudomonas alcaligenes* was more predominant bacteria isolated from imported burger which represented 20%,while *Aeromonas sobria* isolated from local burger was prevalent with rate of occurrence 28.5%, in

minced meat samples *Pseudomonas putida, Lactococcus lacts* and *Kocuria rosea* are predominant bacteria (14%) for each one, *Enterobacter cloacae complex* represented (18.7%) in kebab samples , from imported sausage *Aerococcus urinae* and *Pseudomonas alcaligenes* were more predominant bacteria (25%) while *Pseudomonas putida* was the common bacteria (29%) in local sausage samples .Finally from imported shawarma samples *Staphylococcus equorum, Pseudomonas putida* and *Pseudomonas oryzae* represented the highest isolated bacteria (18.7%) for each one ,while *Kocuria rosea* was dominant bacteria in local samples (22.2%).

Ahmed and Sabiel (2016) worked on frozen meat product (minced meat , burger ,shawarma and sausage) ,showed that *Proteus mirabilis* was isolated from all products of beef meat examined which could be attributed to the use of contaminated water for washing and cleaning during processing , from minced meat *Lactobacillus* spp. and *Kocuria kristinae* were the most isolated organisms (32%), *Kocuria kristinae* was the most isolated bacteria from burger (16%) followed by *Bacillus badius* and *Enterobacter gergoviae* (12%) for each one , these bacteria are found in sausage too with same percentage but the *Staphylococcus capitis* was the predominant bacteria (24%),finally in Shawerma samples lesser bacteria were isolated compare to other meat products with highest isolation rate (24%) of *Bacillus licheniformis* which could be due to environmental contaminations.

Sadeq and his team (2018) worked on isolation and identification of some bacteria from imported meat (beef burger) by using Vitek2 system ,they found that more predominant agent is *E.coli* with percentage 55%, they also found many of bacteria like *Klebsiella pnemoniae, Proteus mirabilis, Staphylococcus aureus, Enterococcus faecium and Serratia marcescens* with 38%, 13%, 44%, 8% and 3% respectively.

Shaltout and his colleagues (2017) mentioned that *E.coli* represented 40% from beef burger samples ,while *Staphylococcus aureus* represent 60% of isolated bacteria .Moreover they found only one species of *Psuedomonas* (*P. aeruginosa*) and failed to detect *Aeromoneus* species from all examined frozen beef burger samples, they

attributed the cause of the presence of bacteria to mishandling and the negligence of hygienic aspects that effected on human public health.

Saad *et al*. (2018) indicated that the microbiological quality of retailed minced meat samples were unsatisfactory, and have health hazards for consumers as it may be lead to food poisoning, these due to the high content of coliform bacteria and *Staphylococcus* sp. especially *aureus* where they attributed the reason for its high rate of contamination during processing from the hands, knives, worker's clothes, the hide or from the environment resulting in an inferior or even unfit quality for human consumption.

Karoki and his team (2018) isolated five genera of bacteria, *Staphylococcus* spp. at 50.4%, *Bacillus* spp. at 19.5%, *Streptococcus* spp. 9.8%, *Proteus* spp. 2.4%, and *E.coli* spp. at 1.6%,from African sausage samples.

The current study the percentage of bacterial contaminants in local meat was less than imported meat that may be due to the fact that local meat is less exposed to contamination, manual handling and exposure to external conditions, in addition to the majority of consumers prefer fresh slaughtered meat and therefore are less exposed to bacterial infection. As for imported meat, this is possible due to several reasons: the imported meat is of origin and sanitary measures were taken followed by production processes that follow international health conditions, poor transportation, storage and manual handling, and the display away from health conditions that may extend for long periods is one of the main pollution factors, if the meat unknown origin and the sanitary measures the production processes used in the production process do not follow the international health conditions, this will lead to the arrival of spoiled goods even if they are transported, storage, manual processing and display within the sanitary conditions, quality control departments and their important role in completing their entry of goods that are considered safe and fit for consumption that are within international health conditions, finally the methods of slaughter whether they are halal or not ,there are many studied about this reason for examples,

Hakim and his team (2020) studied the effect of halal and non-halal slaughtering methods on bacterial contamination of poultry meat ,their study findings suggest that non-halal slaughtered chickens could contain higher bacterial counts that can caused to shorter shelf life and the higher bacterial count is mostly due to more retained blood in the muscle.

Ibrahim *et al*. (2014) and Martuscelli *et al*. (2020) indicated that halal slaughtering method caused to lowering the various microbial loads of poultry meat although the raw meat as well as its product like sausage were contaminated with different microbial species which could reflect contaminated supply, cross contamination, and/or poor hygiene practices.

The results of current study in table (4-3) ,figure (4-3) showed the type of bacteria that isolated from poultry cuts and products which diagnosed by Vitek2 system .

In our study we found *Staphylococcus lentus* and *Staphylococcus vitulinus* are the predominant bacteria in imported and local livers samples (24.2%, 27.1%) respectively, from imported and local thigh samples *Sphingomonas pacumbilis* represented high percentage of detected bacteria(14.2%,13.3%) , high percentage of bacteria *Granulicatella adicans* and *Staphylococcus lentus* (28.5%,21.4%) were reported respectively from imported breast samples while *E.coli* ,*Staphylococcus lentus* and *Granulicatella adicans* represented predominant bacteria that isolated from local breast samples (14.1%)for each one . *Staphylococcus lentus* and *Sphingomonas pacumbilis* revealed high rate of bacteria that isolated from imported and local burger(28.2%,35.7%) respectively, finally kebab samples had high percentage of bacteria *Staphylococcus vitulinus* (18.7%) respectively.

Ahmed and his team (2020) worked on isolated *Staphylococcus* sp. from poultry meat and human swabs by using Vitek2 system ,they isolated 17 species from poultry samples(35) the highly identified species were *Staphylococcus aureus* (14.3%) followed by *Staphylococcus lentus* (8.5%), whereas the least species were *Staphylococcus lugdunensis*, *Stapylococcus simulans* and *Staphylococcus capitis* (2.86% for each).

Sultana and her team (2014) worked on different types of frozen beef and poultry meat products, where many bacterial species were isolated with different percentages(*Staphylococcus* spp. 24.56% - *Alcaligenes* spp.17.54%- *Klebsiella* spp. 12.28%- *Shigella* spp. 8.77%- *Pseudomonas* spp. 8.77%- *Haemophilus* spp. 7.01%- *Micrococcus* spp. 5.26%- *Salmonella* spp. 3.51%- *Corynebacterium* spp. 3.51%- *Enterobacter* spp.3.50%- *Enterococcus* spp. 1.75%- *Actinobacillus* spp.1.75% *and* 1.75%were *Proteus* spp.). They interpreted their results that samples of frozen foods of animal origin that were ready for cooking are responsible for the development of bacterial resistance with a risk to human health and the environment and should be monitored regularly integrated .

The results of the current study, after the preliminary examinations and the Vitek2 compact system, showed the emergence of new types of uncommon bacterial genera in large numbers in frozen meat and poultry samples. The reason may be due to the occurrence of genetic mutations that enabled them to be present in frozen foods, their ability to resist low temperatures as well as the contaminant surrounding the manufacturing process, which enabled them to transfer to meat like the tools and places of slaughter, storage, clothes of workers and their personal hygiene.

5.1.4. Detection of Bacteria by Molecular Techniques:-

5.1.4.1. Multiplex Polymerase Chain Reaction (m-PCR):-

Staphylococcus aureus, Salmonella spp., *Escherichia coli* O157:H7, *Listeria monocytogenes,Vibrio cholerae,* and *V. parahaemolyticus* are the main pathogens involved in food poisoning , Some of these foodborne pathogens can cause life-threatening diseases to humans and animals, whilest all are well recognized. Some are considered emerging but they have recently become more common (Laboratório, 2006).

Salmonella spp., *Staphylococcus aureus, E. coli* O157: H7 and *L.monocytogenes* are the predominant bacteria species that cause public health problems worldwide. In addition, *V. cholerae* is a common bacterium in raw or under processed seafood which

can cause very severe diseases, and has been endemic in Asia and Africa for years. *V. parahaemolyticus* is also a frequent cause of food poisoning in seafood, thus *V. cholerae* and *V. parahaemolyticus* are significant pathogens that require testing in seafood or related products . Lei and his team (2008) detected these species in food samples by using multiplex PCR with special primers and they got positive results .

In our current study, the presence of these common species was screened in all the samples used, and the results of the preliminary examinations as well as the Vitek2compact system proved the absence of any of these species in the examined samples. To confirm this ,multiplex PCR technique was used to investigate their presence using special primers((*stx*) gene of *Escherichia coli* O157: H7, the hemolysin (*hly*) gene of *Listeria monocytogenes*, the invasion (*invA*) gene of *Salmonella* spp., the cholera toxin (*ctx*) gene of *Vibrio cholerae*, the thermolabile hemolysin (*tlh*) gene of *V. parahaemolyticus*, and the thermostable nuclease (*nuc*) gene of *Staphylococcus aureus* , and it's gave negative results, confirming the absence of these species.

The absence of these species may be due to the subjection of frozen food samples to health control systems that reject foods containing these species if they exceed the natural limits, or may be return to the number of samples used in the study for example: Ahmed and his team (2017) worked on isolated of *Listeria monocytogenes* from minced meat, frozen chicken and cheese, from total of 150 samples tested, 13.3% presumptively positive by culturing method while 8% were confirmed to be positive for *L.Monocytogenes* by means of PCR.

Hassanein *et al.* (2011) detected and identified *Salmonella* species in minced beef and chicken meats by using Multiplex PCR, from the total of 75 meat samples examined, *Salmonella* was detected in 5 (20%) of minced frozen beef, 9 (36%) of frozen chicken leg and 13 (52%) of frozen chicken fillet samples analyzed while from the total 30 *Salmonella* positive samples from all examined samples, only five selected *Salmonella* isolates were further identified using multiplex PCR (m-PCR).

Therefore, our results do not agree with the results of several studies that showed the presence of these species in food samples ,whether beef and poultry meat ,such as

El-Malek *et al.* (2010) ; AL-Jobori and AL-Bakri, (2015); Azwai *et al*. (2016); Tao *et al*. (2020); Azinheiro *et al*. (2020); Abdulrahman, (2020) and Yassin , (2021) and agree with some studies that provide the absence of some types of common bacteria like: Elnawawi and his colleagues (2012) isolate some species of entero pathogens bacteria from imported frozen meat, imported frozen chicken and locally frozen chicken while *E. coli O157:H7* and *Salmonella* species failed to be isolated in all examined samples.

Bezerra *et al*. (2010) studied the microbiological quality of hamburgers and isolated many types of bacteria like *Bacillus* spp., *Staphyloccocus* spp.,but *Salmonella* spp. was not detected in any of the samples analyzed.

5.1.4.2: Monoplex Polymerase Chain Reaction (PCR) of 16S r RNA Primers :-

Polymerase chain reaction (PCR) is a useful technique that has revolutionized molecular biology research and it has important application in the diagnosis of microbial infections and genetic diseases, as well as in detection of pathogens in food samples Armany *et al.* (2016).

The results of present study in table (4-4) showed the diagnoses of selected bacteria by amplification of 16S r RNA genes using monoplex PCR.

There are many researches used universal primers (16S rRNA) for the confirmatory diagnosis of bacterial species that were isolated from frozen meat and poultry products samples after their initial diagnosis (Gwida *et al*. 2014 ; Madoroba *et al*. 2021; Delpiazzo *et al*. 2021).

The results in table (4-6) shows the differences in the diagnosis of selected bacteria between Vitek2compact system and 16SrRNA sequences, some species of bacteria appeared in same diagnosis by Vitek2 system and 16SrRNAsequencing while the other species appeared in different diagnosis and some didn't diagnosed by viteck2system and diagnosed by16SrRNAsequencing.The difference in diagnosis may be due to the fact that the database in the Vitek2 system is not constantly updated, and the convergence of some bacterial species in the biochemical tests that the Vitek2

system relies on for diagnosis may be give a different result than the molecular diagnosis.

Rudolph and his colleagues (2019) studied the comparison of Vitek2, MALDI MS, 16S r RNA gene sequencing, and whole-genome sequencing for identification of *Roseomonas mucosa*, they found Both MALDI-TOF MS and 16S r RNA gene sequencing confidently identified the species,while when using the VITEK 2 technique, isolates were misidentified as *Roseomonas gilardii, Rhizobium radiobacter, or Sphingomonas paucimobilis* .

Wu *et al*. (2021) they did comparison of 5 commercial detection systems for *Burkholderia pseudomallei*. The organism was misidentified by the VITEK 2 Compact, Phoenix, VITEK and API 20NE mass spectrometry systems but was ultimately identified by the Bruker Biotyper system and 16S r RNA sequencing.

Al-Kharousi and his team (2016) worked on isolation of opportunistic Pathogens of bacteria from Fresh Fruits and Vegetables ,and identified by molecular (PCR) and biochemical methods Vitek2,some species of isolated bacteria misidentified by Vitek2 system they attribute that to their similarities based on biochemical reactions and not included in the most updated version 7 of the Vitek2 identification database.

Otto-Karg *et al*. (2009)and Dhaegheem, (2021) they found differences in diagnoses of some species of bacteria between vitek2compact system and 16S rRNA sequencing .

***Bacillus licheniformis* :-**

Is a bacteria commonly found in the soil , it is found on bird feathers especially chest and back plumage, and most often in ground-dwelling birds like sparrows and the aquatic species like ducks. *Bacillus licheniformis* is a Gram-positive, spore-forming bacteria widely distributed as a saprophytic organism in the environment and it is a facultative anaerobe, which may allow it to grow in additional ecological niches (Rey *et al*., 2004).

Mikkola and his team (2000) They isolated toxins from three strains *of Bacillus licheniformis* associated with fatal food poisoning and their structures were elucidated. Toxins were purified from methanol extracts of *Bacillus licheniformis* biomass using sperm cells from pigs as an indicator of toxicity. A toxins have been identified as lichenysin A, a cyclic lactonic peptide where the main 3-hydroxy fatty acid is 13-15 carbon atoms long. Thus, the toxins produced by food and food poisoning isolates of *B. licheniformis* were identical to lichenysin A in both the structure and toxicity caused by the sperm of pigs and they found this toxin implicated in the case of a child fatality associated with infant feed (formula). Also it can cause foodborne illness with nausea, vomiting, diarrhea and stomach cramps occurring(5–12 h)after consumption of a variety of foods included , deserts ,ice cream, meat pies and sandwiches where made ranged from(3×10^5 to1×10^8CFU g^{-1}) of the implicated food (Salkinoja-Salonen *et al*. 1999 and Logan, 2012).

***Klebsiella pneumoniae*:-**

It is a Gram negative, encapsulated ,non-motile bacterium which is a member of the enterobacteriaceae family, spread in the natural environment and benignly colonizes the gastrointestinal tracts of healthy humans and animals (Brisse *et al*., 2009) .

Gabida and his team (2015) reported that *K. pneumoniae* is also an opportunistic pathogen because it had capable of causing a wide range of diseases in humans and other animal species,while *K. pneumonia* has been associated with classic foodborne disease outbreaks, it is most notorious for causing extra intestinal human infections like pneumonia, pyelonephritis, , cystitis, septicemia, and pyogenic liver abscess(Shon *et al*., 2013).

It causes a wide array of extra intestinal infections in other animal species and is an important cause of mastitis in dairy cows, notably, the prevalence of antibiotic resistance is increasing among enterobacteriaceae, including *K. pneumonia* which

causing infections in humans and other animal species (Botrel *et al*., 2010 ; Davis and Price, 2016).

***Acinetobacter baumannii* :-**

It is a Gram-negative coccobacillus bacterium which considered as one of the recently emerged *Acinetobacter* species in many parts of the world (Tavakol *et al.*, 2018). It can be recognized in various foodstuffs as fruits, raw vegetables, raw milk, milk products and meat product (Almasaudi, 2018).

Elbehiry and his colleagues (2021) studied the role of raw meat as a reservoir for *A. baumannii*, and the results of their research showed the majority of *A. baumannii* strains illustrated strong antimicrobial resistance against amoxicillin/clavulanic acid, gentamicin, ampicillin ,tetracycline, and tobramycin. So, the existence of multidrug-resistant *A.baumannii* in meat may represent a clear threat to human health.

***Aeromonas veronii* :-**

It is a Gram-negative, facultative anaerobic and rod-shaped bacterium, which is widely distributed in nature with strong environmental adaptability (Parte, 2014).

Ghenghesh *et al*. (1999) and Zhang and his team (2019) reported that *A. veronii* can infect birds, freshwater fish, amphibians and red meat animals, resulting in serious losses to the aquaculture industry and threatening food safety. Also, it can cause human infections, especially in elderly and children with low immunity, causing sepsis, gastroenteritis, and other diseases.

In recent years, the development of aquaculture has led to an increase in the incidence of bacterial diseases. the use of antibiotics caused an increase in *Aeromonas* resistance to these antibiotics, and the presence of antibiotic residues in aquatic products threatens human health, reports shown the occurrence of infectious diarrhea and food poisoning for people infected with these bacteria (Stratev *et al*., 2015).

***Enterobacter cloacae* :-**

It is a Gram negative , facultative anaerobic and rod shaped bacterium, occur in food ,water, soil, sewage, and as commensal microflora in the intestinal tracts of animals and humans. They were frequently isolated from clinical and food samples as opportunistic pathogens (Lund *et al.*, 2000 and Nyenje *et al.*, 2012). Recently, *Enterobacter cloacae* has emerged as drug-resistant bacterial species .the development of antimicrobial resistance among *Enterobacter* spp. including resistance to extended spectrum cephalosporins represent a great concern on human health (Harada *et al.*, 2017). Therefore, isolation of *E. cloacae* strains that are resistant to colistin (an antibiotic polypeptide) still useful against multidrug resistant Gram-negative bacteria such as carbapenem- resistant enterobacteriaceae is critical (Lin and his team, 2017).

However, some studies have shown that certain *E. cloacae* isolated from foods maybe resistant to this drug with the rise in infections due to multidrug resistant gram negative, bacilli, more emphasis has been placed on colistin (Karaaslan *et al.*, 2016).

***Pseudomonas* spp. :-**

Pseudomonas spp. are Gram-negative, aerobic, non-spore-forming bacteria ,it is the most common bacteria implicated in spoilage, especially of refrigerated food. Also recognized to be able to colonize environmental production as well as equipment and facilities for long periods because their ability to produce persistent biofilms (Spanu *et al.*, 2018 and Rossi *et al.*, 2018).

The *pseudomonas* genus induces more than 140 species and represents the most psychrotrophic bacteria which are highly Proteolytic and or strong lipolytic leading to biological changes in the composition of meat and meat products particularly at low temperature (Gill and Newton, 1982 and Ibrahim *et al.*, 2016).

Ibrahim and his team (2016) studied the Prevalence and molecular characterization of *Pseudomonas* species in frozen imported meat and from there previous results they found *P. fluorescence and P. alcaligenes* represented major species, which could be

isolated, this may reflect its resistance against many stress factors such as water activity, low temperature and inhibitory action of carbon dioxide.

***Kocuria* spp. :-**

The genus *Kocuria* includes Gram-positive, catalase-positive, coagulase-negative cocci, belonging to family micrococcaceae, the genus includes 26 species with *Kocuria varians* and *Kocuria rosea* as its major representatives and the youngest species, *K. coralli* has recently been described in 2020 (Li and Zhang, 2020). *Kocuria* spp. can be found in diverse environments such as skin of mammals, milks, dairy products , seafood , fermented and unfermented meat products of various origins (Noor Uddin *et al*., 2013; Kandi *et al*., 2016; Uzair *et al*., 2018; Machado *et al*., 2020).

Savini *et al*. (2010) and Kandi *et al*. (2016) reported that it is known that the prevalence of these infections is underestimated since phenotypic identification methods frequently used in clinical analysis can mistakenly identify *Kocuria* isolates as coagulase-negative *Staphylococcus* spp. based on their Gram staining, coagulase and catalase characteristics. Similarly, assessment of these species in foods might be difficult.

Gardini and his team (2003) reported that the strains of *Staphylococcus* and *Kocuria* resistant to antibiotics (some multi-resistant) isolated from fermented sausages in Italy, and recommended careful use of antibiotics in animal rearing and improvement in hygienic conditions during livestock production.

Francisella tularensis :-

It is a Gram-negative ,facultative, intracellular bacterium that causes the disease tularemia, also known as deer fly fever or rabbit fever (Kingry and Petersen, 2014). *F. tularensis* is classified by the United States Select Agent Program as a Tier 1 select agent because it is highly infectious, potentially lethal, and easily disseminated through multiple routes including through the bite of an infected vector, through the air, or through contact with contaminated water, soil or meat. Symptoms depend on

the method of infection but typically include fever and lymphadenopathy and may progress to the much more serious pneumonic or typhoidal tularemia. Both of these forms may have a case fatality rate of greater than 40% if untreated (Roth, 2016).

***Serratia* spp.:-**

It is a Gram-negative bacterial genus that belongs to the family of enterobacteriaceae. This genus is composed of 22 species which can be found in various environments and hosts such as insect, plant, soil, water, and food raw materials (Parte *et al.*, 2020). They are associated with food spoilage a real economic and environmental issue resulting in food losses and waste within the food supply chain n (Gustavsson *et al.*, 2011). The genetically closely related food species *S. liquefaciens, S. quinivorans, S. proteamaculans* and *S. grimesii* ,which can grow at low temperature, under hypobaric conditions and in CO2-enriched anoxic atmosphere (Schuerger *et al.*, 2013; Schuerger and Nicholson, 2016) thus prevalent in food products whether vacuum or modified atmosphere packaging is used, their spoilage activities can be associated with gas production in meat products (Chaves *et al.*, 2012).

***Lactic acid bacteria*:-**

They are a group of Gram-positive bacteria, including species of *Lactobacillus, Oenococcus, Leuconostoc* and *Pediococcus*, some of which are useful in producing fermented foods. However, under low temperature, low oxygen and acidic conditions, these bacteria become the predominant spoilage organisms on a variety of foods, for examples it's caused greening of meat and spoilage of canned or packaged meat and vegetables. *Lactic acid* bacteria may also produce large amounts of an exo polysaccharide that causes slime on meats (Rawat, 2015).

***Escherichia coli* (*E. coli*) :-**

It is a Gram –negative bacteria, a member of the enterobacteriaceae family and it is a normal inhabitant of the gut of poultry and frequent microbial contaminant of retail

poultry meat (Davis *et al*., 2018). Skurnik *et al*. (2006) reported that *E. coli* is also known as one of the most important foodborne pathogens in humans which may be associated with a diversity of acute and invasive infections in humans and it's easily to be disseminated in different ecosystems through the food chain.

Addis and Sisay (2015) explained that chicken meat is generally contaminated by *E. coli* during handling, cleaning, improper dressing and unhygienic practices of selling meat. Contaminated chicken meat can be considered as a potential source of infection with *E. coli*, either by direct contact during food preparation or by consumption of undercooked or raw meat products.

Garcia-Hernandez and his team (2015) reported that several strains of *E. coli* became resistant to heat by the addition of salt, about 2% of *E. coli* including food isolates harbor heat resistant genes and show increased heat resistance. The symptoms of food poisoning because of *E. coli* are abdominal cramps, vomiting, in some cases, bloody diarrhea in humans. Sometimes, the infection caused by *Shiga* toxin-producing by *E. coli* may be lead to hemolytic–uremic syndrome that cause kidney failure (Switaj *et al*., 2015).

***Lysinibacillus boronitolerans*:-**

Is a spore-forming ,Gram-positive, motile, rod-shaped and boron-tolerant bacterium, it is commonly found in soil and has been isolated from plant tissues , from fermented plant seed products , and even from puffer fish liver specimens . And used as an insecticidal microorganism (Ahmed, 2007).

***Psychrobacter sanguinis* :-**

Non-haemolytic, non-motile, Gram-negative coccobacillus (0.5–1.0 µm wide and 1.0–2.0 µm long). Colonies are 1–2 mm in diameter, moist, non-pigmented, circular and smooth with entire margins. Grows at 4–37 °C (optimum 30–37 °C) (Wirth *et al.*, 2012). *P. sanguinis* is a new species described in 2012; the type strain was isolated from the blood of an 84-year male in the United States ,because *P. sanguinis* is a

recently described species, data are lacking concerning its pathogenicity. It was first isolated from the blood cultures of 4 patients in New York ,however, it is probably an environmental bacteria and was recovered from seaweed samples in India (Wirth *et al*., 2012).

Comamonas testosterone :-

It is aerobic, gram-negative, motile, pink-pigmented, oxidase-positive bacilli that grow well on routine bacteriological media. It has capable of utilising testosterone, 4-hydroxybenzoate, acetate, and lactate as their sole carbon sources, but not glucose and most of the carbohydrates, hence the name "testosteroni" (Liu *et al*., 2015). *C. testosteroni* can be found in intravenous catheters, the respiratory tract, the abdomen and urinary system, and the central nervous system (Altun *et al*., 2013).

Methylogaea oryzae **:-**

Is grows on methane or methanol as its sole carbon and energy source. It is a Gram-negative, nonmotile, nonpigmented, . It is aerobic, neutrophilic, and mesophilic (optimum growth at 30–35 °C) (Geymonat *et al*., 2011).

CONCLUSIONS

&

RECOMMENDATION

Conclusions:-

1- The study displayed that frozen foods (meat products and chicken cuts and their products) contain many bacteria , although local products were contaminated lower than imported ones.

2- The contamination might occurs inside slaughter houses, during the additional processing of meat , the tools used and workers or during storage its. Therefore ,it is important to improve the health quality of meat(ensure that it is fit for human consumption) ,whether its imported or local .

3- Isolation and identification of many pathogenic bacteria such as *Bacillus licheniformis, Klebsiella pneumonia* and *Acinetobacter baumannii* which cause great public health concern due to its zoonotic transmission to humans and it is ability to cause severe disease in human.

4- Isolation and identification of many spoilage bacteria such as *Psuedomonas* spp., *Serratia* spp. and *Lactic acid* bacteria that cause spoilage of food by changing its taste and smell , which may lead to economic losses.

5- Sixteen bacterial strains isolated from frozen foods were recorded in the Gene Bank as a new strains recorded for the first time in Iraq.

Recommendations:-

1- Regular monitoring of meat and meat products is necessary to prevent potential public health problems, and the need to improve food safety by implementing sanitary measures at all levels from production to consumption.

2- The lack of borders control, entry of most shipments of frozen meat unchecked, frequent electricity shortages for long hours, poor storage all contribute to increasing contamination with foodborne pathogens. Therefore ,food shipment should be check before entering the country, don't allow for unreliable sources to import and improve storage conditions.

3- The proper management should be taken in the markets to reduce the transmission of zoonoses to humans, and an appropriate control program for microbial contamination of all foodborne pathogens should be established.

4- Studying the effects of environmental conditions on proliferation of meat spoilage microorganisms .

5- A comparative study of meat contamination before and after its cooking process.

6- Conducting a molecular study of the isolated bacterial species and determining the degree of their resistance to antibiotics.

7- Using a Multiplex PCR technique to diagnose isolated bacterial species using special primers for each type.

8- PCR technique the best for identification of bacterial and they were isolates than the Vitek2 compact system.

9- The advantages of Monoplex PCR and Multiplex PCR techniques over traditional methods of biochemical testing. Moreover, traditional methods should be replaced by advanced molecular methods, because they are reliable, fast, sensitive, and labor saving.

10- Study of large number of samples from different sources of local and imported products.

REFERENCES

References:-

Abbas A, A., and Samar Y, E. S. (2014). Microbiological criteria of some meat products. *Alexandria Journal of Veterinary Sciences* [AJVS],42 (1), 83-89.

Abbas, B. A., and Alghanim, A. M. (2016). Detection of virulence genes in *Escherichia coli* isolated from frozen meat in Basrah market. *Basrah Journal of Veterinary Research*, 15(3), 134-138.

Abd El Tawab, A. A., El-Hofy, F. I., Maarouf, A. A., and El-Said, A. A. (2015). Bacteriological studies on some food borne bacteria isolated from chicken meat and meat products in Kaliobia Governorate. *Benha Veterinary Medical Journal*, 29(2), 47-59.

Abdulrahman, R. F. (2020). Detection of *Staphylococcus aureus* from local and imported chicken in duhok province/kurdistan region of iraq using conventional and molecular methods. *Basrah Journal of Veterinary Research*, 19(1),134-146.

Abubakar, I., Irvine, L., Aldus, C.F., Wyatt, G.M., Fordham,R., Schelenz, S., Shepstone, L., Howe, A. et al. (2007). A systematic review of the clinical, public health and cost effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. *Health Technology Assession*, 36, 1–216.

Addis, M., and Sisay, D. (2015). A review on major food borne bacterial illnesses. *Journal of Tropical Diseases & Public Health*,3(4),1-7.

Adwan, G. M.;Alqarem, B. R. ,and Adwan, K. M.(2015) . Prevalence of food-borne pathogens in meat samples in Palestine. *International Food Research Journal*, 22(5): 1806-1812.

Ahmed, A. A., and Sabiel, Y. A. (2016). Detection of microbial contamination of processed beef meat by using Api strips and automated Vitek2 compact system. *Microbiology Research Journal International*, 13(2),1- 8.

Ahmed, I., Yokota, A., Yamazoe, A., and Fujiwara, T. (2007). Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to

Lysinibacillus sphaericus comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 57(5), 1117-1125.

Ahmed, M., Ahmed, H., and Al-azeem, A. (2020). Molecular detection of antibiotic resistance and virulence genes in *staphylococcus* species isolated from human and poultry. *SVU-International Journal of Veterinary Sciences*, *3*(1), 100-122.

Ahmed, S. M., Hall, A. J., Robinson, A. E., Verhoef, L., Premkumar, P., Parashar, U. D., ...and Lopman, B. A. (2014). Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *The Lancet infectious diseases*, 14(8), 725-730.

Ahmed, S. S. T. S., Tayeb, B. H., Ameen, A. M., Merza, S. M., and Sharif, Y. H. M. (2017). Isolation and molecular detection of *Listeria monocytogenes* in minced meat, frozen chicken and cheese in Duhok province, Kurdistan region of Iraq. *Journal Food Microbiol Saf Hyg*, *2*(118), 2476-2059.

Akeda, Y. (2015). Food safety and infectious diseases. *Journal of nutritional science and vitaminology,* 61(Supplement), S95-S95..

Alert, O. (2014). A review of foodborne illness in America from 2002-2011. *Center for Science in the Public Interest: Washington, DC, USA.*

Al-jasser, M. S. (2012). Effect of cooling and freezing temperatures on microbial and chemical properties of chicken meat during storage. *JOURNAL OF FOOD AGRICULTURE & ENVIRONMENT*, 10(1), 113-116**.**

AL-Jobori, K. M., and AL-Bakri, A. K. (2015). Comparison between PCR and culture methods for detection of *Salmonella Typhimurium* from food and beverage *Donnish Journal of Food Science and Technology*, 1(2),006-016.

Al-Kharousi, Z. S., Guizani, N., Al-Sadi, A. M., Al-Bulushi, I. M., and Shaharoona, B. (2016). Hiding in fresh fruits and vegetables: opportunistic pathogens may cross geographical barriers. *International Journal of Microbiology* ,Volume 2016, Article ID 4292417, 14 pages.

Almasaudi, S. B. (2018). *Acinetobacter* spp. as nosocomial pathogens: Epidemiology and resistance features. *Saudi journal of biological sciences*, 25(3), 586-596.

Alonso-Calleja, C., Martínez-Fernández, B., Prieto, M., and Capita, R. (2004). Microbiological quality of vacuum-packed retail ostrich meat in Spain. *Food Microbiology*, 21(2), 241– 246.

AL-Rawi, K. M., and Khalf Allah, A. M. (2000). Design and analysis of agricultural experiments. University of Mosul. Ministry of Higher Education and Scientific Research. Dar Al Kuttab for printing and publishing . Mosul .Iraq.

AL-Tamimi, A. H., and AL-Khafaji, N. J. (2021). Comparative study between imported frozen and local fresh poultry's meat in respect of Bacterial contamination with enterobactericeae in Diyala province. *Diyala Journal for Veterinary Sciences*, 1(1), 1-18.

Altun, E., Kaya, B., Taktakoğlu, O., Karaer, R., Paydas, S., Balal, M., and Seyrek, N. (2013). *Comamonas testosteroni* peritonitis secondary to dislocated intrauterine device and laparoscopic intervention in a continuous ambulatory peritoneal dialysis patient. *Peritoneal Dialysis International*, *33*(5), 576-578.

Armany, G. A., Ahmed, H. A., Ibrahim, H. M., and Amin, R. A. (2016). Detection of some foodborne pathogens in meat products by Polymerase Chain Reaction. *Benha Veterinary Medical Journal*, 30(1), 323-330.

Asghar, A. (1988). Perspectives on warmed-over flavor. *Food Technology*, 42, 102-108.

Ayres,J.C,Mundt, J.D. and Sandine; W.E. (1980) .Microbiology of food. San Francisco ;USA; W.H.Freeman and Co.

Azinheiro, S., Carvalho, J., Prado, M., and Garrido-Maestu, A. (2020). Multiplex detection of *Salmonella* spp., *E. coli* O157 and *L. monocytogenes* by qPCR melt curve analysis in spiked infant formula. *Microorganisms*, 8(9), 1359.

Azwai, S. M., Alfallani, E. A., Abolghait, S. K., Garbaj, A. M., Naas, H. T., Moawad, A. A., ... and Eldaghayes, I. M. (2016). Isolation and molecular identification of *Vibrio* spp. by sequencing of 16S rDNA from seafood, meat and meat products in Libya. *Open veterinary journal*, 6(1), 36-43.

Beveridge, T. J. (2001). Use of the Gram stain in microbiology. *Biotechnic and Histochemistry*, *76*(3), 111-118.

Bezerra, A. C. D., Reis, R. B. D., and Bastos, D. H. M. (2010). Microbiological quality of hamburgers sold in the streets of Cuiabá-MT, Brazil and vendor hygiene-awareness. *Food science and technology*, 30(2), 520-524**.**

Biesalski, H. K. (2005): Meat as a component of a healthy diet. Are there any risks or benefits if meat is avoided in the diet. *Meat Science*, 70: 509-524.

Blackburn, C. D. W. (2006). Managing microbial food spoilage: an overview. *Food spoilage microorganisms* ,Wood head Publishing Ltd, Cambridge/UK,p 147-170.

Bohaychuk, V. M., Gensler, G. E., King, R. K., Manninen, K. I., Sorensen, O., Wu, J. T., ... and McMullen, L. M. (2006). Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail marketplace in Edmonton, Alberta, Canada. *Journal of Food Protection*, 69(9), 2176-2182.

Borch, E., Kant-Muermans, M. L., and Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology*, 33(1), 103– 120.

Botrel, M. A., Haenni, M., Morignat, E., Sulpice, P., Madec, J. Y., and Calavas, D. (2010). Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhône-Alpes, France. *Foodborne pathogens and disease*, 7(5), 479-487.

Boziaris, I. S., and Parlapani, F. F. (2017). Specific spoilage organisms (SSOs) in fish. *In The microbiological quality of food* (pp. 61-98).

Brisse, S., Fevre, C., Passet, V., Issenhuth-Jeanjean, S., Tournebize, R., Diancourt, L., and Grimont, P. (2009). Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. *PloS one*, 4(3), e4982.

Carrizosa, E., Benito, M. J., Ruiz-Moyano, S., Hernández, A., del Carmen Villalobos, M., Martín, A. and de Guía Córdoba, M. (2017). Bacterial communities of fresh goat meat packaged in modified atmosphere. *Food microbiology*, 65, 57-63.

Caruso, I. P., Panwalkar, V., Coronado, M. A., Dingley, A. J., Cornélio, M. L., Willbold, D., Arni, R.K., and Eberle, R. J. (2017). Structure and interaction of *Corynebacterium pseudotuberculosis* cold shock protein A with Y-box single-481 stranded DNA fragment. *Federation of European Biochemical Societies Journal, 285*(2), 372-390.

Casaburi, A., Piombino, P., Nychas, G. J., Villani, F., and Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology*, 45, 83– 102.

Chamberlain, J. S., Gibbs, R. A., Rainer, J. E., Nguyen, P. N., and Thomas, C. (1988). Deletion screening of the duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic acids research*, 16(23), 11141-11156.

Chaves, D., Hsu, J., Li, F., and Shakernia, O. (2012). Efficient algorithms for computing risk parity portfolio weights. *The Journal of Investing*, 21(3), 150-163.

Chellaiah, R., Shanmugasundaram, M., and Kizhekkedath, J. Advances in meat preservation and safety.(2019). *International Journal of Science and Research (IJSR)*, Volume 9 Issue 3,1499-1502.

Chen, J., Tang, J., Liu, J., Cai, Z., and Bai, X. (2012). Development and evaluation of a multiplex PCR for simultaneous detection of five foodborne pathogens. *Journal of applied microbiology*, 112(4), 823-830.

Collee, J. G., Fraser, A. G., Marmion, B. P., and Simmons, A. (1996). Mackie and mccartney. *Practical medical microbiology*, 14, 413-424.

Darbandi, F. (2011). Parallel comparison of accuracy in Vitek2 auto analyzer and API 20 E/API 20 NE microsystems **,** phD. Thesis, University College of Borås, School of Engineering,pp52.

Davis, G. S., and Price, L. B. (2016). Recent research examining links among *Klebsiella pneumoniae* from food, food animals, and human extraintestinal infections. *Current environmental health reports*, 3(2), 128-135.

Davis, G. S., Waits, K., Nordstrom, L., Grande, H., Weaver, B., Papp, K., ... and Price, L. B. (2018). Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. *BMC microbiology*, *18*(1), 1-7.

DeCory, T. R., Durst, R. A., Zimmerman, S. J., Garringer, L. A., Paluca, G., DeCory, H. H., and Montagna, R. A. (2005). Development of an immunomagnetic bead-immunoliposome fluorescence assay for rapid detection of *Escherichia coli* O157: H7 in aqueous samples and comparison of the assay with a standard microbiological method. *Applied and environmental microbiology*, 71(4), 1856-1864.

Delpiazzo, R., Barcellos, M., Barros, S., Betancor, L., Fraga, M., Gil, J., ... and Calleros, L. (2021). Accurate and fast identification of *Campylobacter fetus* in bulls by real-time PCR targeting a 16S rRNA gene sequence. *Veterinary and animal science*, 11, 100163.

Dhaegheem, H., Naeem.(2021). Identification of petroleum biodegradable bacteria isolated from contaminated soils in Misan Province / Iraq.(Thesis, University of Misan).

Dimitrijević, S. I., Mihajlovski, K. R., Antonović, D. G., Milanović-Stevanović, M. R., and Mijin, D. Ž. (2007). A study of the synergistic antilisterial effects of a sub-lethal dose of lactic acid and essential oils from Thymus vulgaris L., Rosmarinus officinalis L. and Origanum vulgare L. *Food chemistry*, 104(2), 774-782.

Dohlen, S., Braun, C., Brodkorb, F., Fischer, B., Ilg, Y., Kalbfleisch, K., Kreyenschmidt, J. (2017). Effect of different packaging materials containing poly-[2-(tert-butylamino) methylstyrene] on the growth of spoilage and pathogenic bacteria on fresh meat. *International Journal of Food Microbiology*, 257, 91– 100.

Doulgeraki, A. I., Ercolini, D., Villani, F., and Nychas, G. J. E. (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. *International Journal of Food Microbiology*, 157(2), 130– 141

Eid, H., Mohamed, G., and El Borolos, I. (2018). Phenotypic and genotypic detection of virulence factors of *Staphylococcus aureus* isolated from meat and meat products. *Suez Canal Veterinary Medical Journal*. SCVMJ, 23(2), 59-79.

Elbasheir, A. R. E., El Ayis, A. A., and Abdelrahim, A. A. (2019). Aerobic bacteria isolated from fresh and frozen chicken's carcasses in Khartoum state, Sudan. *World Journal Of Pharmacy And Pharmaceutical Sciences*, 8(4), 1700-1710.DOI: 10.20959/wjpps20194-13564.

Elbehiry, A., Marzouk, E., Moussa, I. M., Dawoud, T. M., Mubarak, A. S., Al-Sarar, D., ... and Zahran, R. N. (2021). *Acinetobacter baumannii* as a community foodborne pathogen: Peptide mass fingerprinting analysis, genotypic of biofilm formation and phenotypic pattern of antimicrobial resistance. *Saudi Journal of Biological Sciences*, 28(1), 1158-1166.

Elizaquı´vel, P. and Aznar, R. (2008) A multiplex RTi-PCR reaction for simultaneous detection of *Escherichia coli* O157:H7, *Salmonella* spp. and *Staphylococcus aureus* on fresh, minimally processed vegetables. *Food Microbiol* 25, 705–713.

El-Malek, A. M. A., Ali, S. F. H., Hassanein, R., Mohamed, M. A., and Elsayh, K. I. (2010). Occurrence of *Listeria* species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of *Listeria monocytogenes*. *Veterinary World*, 3(8),353-359.

Elnawawi, F. A., Attala, O. A., and Saleh, S. (2012). Enteropathogens of public health importance in imported frozen meat and chicken. *International Journal of Microbiology Research*, 3(1), 59-63.

Ercolini, D., Casaburi, A., Nasi, A., Ferrocino, I., Di Monaco, R., Ferranti, P., ... and Villani, F. (2010). Different molecular types of *Pseudomonas fragi* have the same overall behaviour as meat spoilers. *International journal of food microbiology*, 142(1-2), 120-131.

Ernst, F. G. M., Erber, L., Sammler, J., Jühling, F., Betat, H., and Mörl, M. (2018). Cold adaptation of tRNA nucleotidyl transferases: A tradeoff in activity, stability and fidelity. *RNA Biology*, 15 (1), 144 - 155.

Food and Drug Administration "FDA" (2012): Bad bug book food borne pathogenic microorganisms and natural toxins handbook, 2nd ed. Page: 292.

Food and Drug Administration "**FDA**". (2020). What you need to know about foodborne illnesses. May 9, 2018.

Fratmico PM, B. A., Smith JL. (2005): Foodborne pathogens in microbiology and molecular biology. Caister Academic Press ,Wymondham, Norfolk, UK, page: 273.

Frazier, W. C., and Westhoff, D. C. (1978). Food microbiology 3rd Edition McGraw Hill Book company. *New York*, p,540.

Fukuda, K. (2015). Food safety in a globalized world. *Bulletin of the World Health Organization*, *93*(4), 212-213.

Gabida, M., Gombe, N. T., Chemhuru, M., Takundwa, L., Bangure, D., and Tshimanga, M. (2015). Foodborne illness among factory workers, Gweru, Zimbabwe, 2012: a retrospective cohort study. *BMC research notes*, 8(1), 1-6.

Garcia-Hernandez, R., McMullen, L., and Gänzle, M. G. (2015). Development and validation of a surrogate strain cocktail to evaluate bactericidal effects of pressure on verotoxigenic *Escherichia coli*. *International journal of food microbiology*, 205, 16-22.

Gardini, F., Tofalo, R., and Suzzi, G. (2003). A survey of antibiotic resistance in Micrococcaceae isolated from Italian dry fermented sausages. *Journal of food protection*, 66(6), 937-945.

Gao, H., Zhao, Y., and Liu, C. C. (2014). Pathogenic microorganisms in response to environmental stress of VBNC state and its potential impact on risk assessment. *Microbiology*, 41 (1), 169-177.

Geymonat, E., Ferrando, L., and Tarlera, S. E. (2011). *Methylogaea oryzae* gen. nov., sp. nov., a mesophilic methanotroph isolated from a rice paddy field. *International journal of systematic and evolutionary microbiology*, *61*(11), 2568-2572.

Ghenghesh, K. S., Abeid, S. S., Jaber, M. M., & Ben-Taher, S. A. (1999). Isolation and haemolytic activity of *Aeromonas* species from domestic dogs and cats. *Comparative immunology, microbiology and infectious diseases*, 22(3), 175-179.

Gill, C. O. (2001). Microbial control with cold temperatures. In control of foodborne microorganisms,1st ed (pp. 55-74). CRC Press.

Gill, C. O., and Newton, K. G. (1982). Effect of lactic acid concentration on growth on meat of Gram-negative psychrotrophs from a meatworks. *Applied and Environmental Microbiology*, 43(2), 284-288.

Goldman, E., and Lorrence, H. G. (2009). Practical Handbook of Microbiology. (2nd Ed). Printed in the United Stated of America. 34:1367-1378.

Gounot, A. M. (1986). Psychrophilic and psychrotrophic microorganisms. *Experientia*, 42(11), 1192-1197.

Gour, S., Khare, M., Patidar, R. K., Sahare, K. N., Bagde, S., Chauhan, S., ... and Singh, V. (2014). Screening of microorganisms from different sites of restaurants and dhabas. *International Journal of Pharmaceutical Sciences and Research*, 5(1), 183.

Gwida, M., Hotzel, H., Geue, L., and Tomaso, H. (2014). Occurrence of enterobacteriaceae in raw meat and in human samples from Egyptian retail sellers. *International scholarly research notices*, vol. 2014,1-6.

Gustavsson J, Cederberg C, Sonesson U, Otterdijk RV, Meybeck A (2011) Global food losses and food waste. extent, causes and prevention. *Food and Agriculture Organization of the United Nations, Rome***.** p(1-16)

Hafez, M., Ibrahim, H., and Amin, R. (2019). Assessment of bacterial evaluation of imported frozen meat. *Benha Veterinary Medical Journal*, 37(2), 42-45.

Hakim, L. I., Isa, N. M. M., Tahir, S. M., and Ibitoye, E. B. (2020). Effect of halal and non-halal slaughtering methods on bacterial contamination of poultry meat. *Sains Malaysiana*, 49(8), 1947-1950.

Haleem, A. M., Al-bakri, S. A., and Al-Hiyaly, S. A. (2013). Determination of microbial content in poultry meat in local Iraqi markets. *Journal of Microbiology Research*, 3(6), 205-207.

Hammond, S. T., Brown, J. H., Burger, J. R., Flanagan, T. P., Fristoe, T. S., Mercado-Silva, N., ... and Okie, J. G. (2015). Food spoilage, storage, and transport: Implications for a sustainable future. *BioScience*, 65(8), 758-768.

Hantsiszacharov, E., and Halpern, M. (2007). Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits. *Applied & Environmental Microbiology*, 73 (22), 7162-7168.

Harada, K., Shimizu, T., Mukai, Y., Kuwajima, K., Sato, T., Kajino, A., ... and Kataoka, Y. (2017). Phenotypic and molecular characterization of antimicrobial resistance in *Enterobacter* spp. isolates from companion animals in Japan. *PLoS One*, *12*(3), e0174178.

Hassanien, F. M., El-Sabagh, R. A., Nassief, M. Z., and Refat, M. S. (2016). Bacterial and chemical quality of frozen chicken meat received at governmental hospital modern. *Benha veterinary medical journal*, 30(1), 109-117.

Hassanien, F., Shaltout, F. A. E., Fahmey, M. Z., and Elsukkary, H. F. A. (2020). Bacteriological quality guides in local and imported beef and their relation to public health. *Benha Veterinary Medical Journal*, 39(1), 125-129.

Hassanein, R., Ali, S. F. H., El-Malek, A., Mohamed, A., Mohamed, M. A., & Elsayh, K. I. (2011). Detection and identification of *Salmonella* species in minced beef and chicken meats by using multiplex PCR in Assiut city. *Veterinary World*, 4(1),5-11.

Hatab, S., Athanasio, R., Holley, R., Rodas-Gonzalez, A., and Narvaez-Bravo, C. (2016). Survival and reduction of shiga toxin-producing *Escherichia coli* in a fresh cold-pressed juice treated with antimicrobial plant extracts. *Journal of food science*, 81(8), M1987-M1995.

Höll, L., Behr, J., and Vogel, R. F. (2016). Identification and growth dynamics of meat spoilage microorganisms in modified atmosphere packaged poultry meat by MALDI-TOF MS. *Food Microbiology*, 60, 84– 91.

Hugenholtz, P., Goebel, B. M., and Pace, N. R. (1998). Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of bacteriology*, 180(18), 4765-4774.

Hui, Y. H.; Nip, W. R; Rogers, R. W. and Youn, O. A.(2001). Meat science and application. Marcel Bekker, New York,pp.704.

Húngaro, H. M., Caturla, M. Y., Horita, C. N., Furtado, M. M., and Sant'Ana, A. S. (2016). Blown pack spoilage in vacuum-packaged meat: A review on clostridia as causative agents, sources, detection methods, contributing factors and mitigation strategies. *Trends in Food Science & Technology*, 52, 123– 138.

Hussain, M. A. (2016). Food contamination: major challenges of the future. *Journal of Food*, *5*(2), 21.

Ibrahim, H. M., Bou El-Roos, N. A., and Abd Elsalam, M. (2016). Prevalence and molecular characterization of *Pseudomonas* species in frozen imported meat. *Benha Veterinary Medical Journal*, *31*(2), 220-224.

Ibrahim, S. M., Abdelgadir, M. A., and Sulieman, A. M. (2014). Impact of halal and non-halal slaughtering on the microbiological characteristics of broiler chicken meat and sausages. *Food and Public Health*, 4(5), 223-228.

Ikeda, S., Takabe, K., Inagaki, M., Funakoshi, N., and Suzuki, K. (2007). Detection of gene point mutation in paraffin sections using in situ loop-mediated isothermal amplification. *Pathology international*, 57(9), 594-599.

Iraqi standard specification (No.2270),(2020). Microbiological limits in food part 4 microbiological limits of meat and chicken products. Central Organization for Standardization and Quality Control (COSQC), Ministry of Planning, Iraq.

Jaouen, T., Dé, E., Chevalier, S., and Orange, N. (2004). Pore size dependence on growth temperature is a common characteristic of the major outer membrane

protein OprF in psychrotrophic and mesophilic *Pseudomonas* species. *Applied and Environmental Microbiology*, 70 (11), 6665 - 6669.

Jay, J. M., Loessner, M. J., and Golden, D. A. (2008). *Modern food microbiology*. 7th Edition .Springer Science and Business Media.

Ju, J., Xie, Y., Guo, Y., Cheng, Y., Qian, H., and Yao, W. (2019). The inhibitory effect of plant essential oils on foodborne pathogenic bacteria in food. *Critical reviews in food science and nutrition*, 59(20), 3281-3292.

Kalalou I, Faid M, Ahami AT (2004). Extending the shelf life of fresh minced camel meat at ambient temperature by *Lactobacillus delbruekii* subsp. *delbruekii*. *Electronic Journal Biotechnology*, 7(3),246-251.

Kandi, V., Palange, P., Vaish, R., Bhatti, A. B., Kale, V., Kandi, M. R., and Bhoomagiri, M. R. (2016). Emerging bacterial infection: identification and clinical significance of *Kocuria* species. *Cureus*, 8(8),e731.

Karaaslan, A., Çağan, E., Kadayifci, E. K., Atıcı, S., Akkoç, G., Yakut, N., ... and Bakır, M. (2016). Intravenous colistin use for multidrug-resistant Gram-negative infections in pediatric patients. *Balkan medical journal*, *33*(6), 627-632.

Karoki, W. H., Karanja, D. N., Bebora, L. C., and Njagi, L. W. (2018). Isolation, characterization, and quantification of bacteria from African sausages sold in Nairobi County, Kenya. *International journal of food science*, 2018,p9.

Kaur, M., Shang, H., Tamplin, M., Ross, T., and Bowman, J. P. (2017). Culture-dependent and culture-independent assessment of spoilage community growth on VP lamb meat from packaging to past end of shelf-life. *Food Microbiology*, 68, 71– 80.

Kawasaki, S., Fratamico, P. M., Horikoshi, N., Okada, Y., Takeshita, K., Sameshima, T., and Kawamoto, S. (2009). Evaluation of a multiplex PCR system for simultaneous detection of *Salmonella* spp., *Listeria monocytogenes, and Escherichia coli* O157: H7 in foods and in food subjected to freezing. *Foodborne pathogens and disease*, *6*(1), 81-89.

Kawasaki, S., Horikoshi, N., Okada, Y., Takeshita, K., Sameshima, T., and Kawamoto, S. (2005). Multiplex PCR for simultaneous detection of *Salmonella* spp., *Listeria monocytogenes, and Escherichia coli* O157: H7 in meat samples. *Journal of food protection*, 68(3), 551-556.

Kawasaki, S.; Fratamico, P.M. ; Horikoshi, N.; Okada, Y. ; Takeshita , K.; Sameshima, T. , et al.(2010). Multiplex real-time polymerase chain reaction assay for simultaneous detection and quantification of *Salmonella* species, *Listeria monocytogenes and Escherichia coli* O157:H7 in ground pork samples. *Food-borne Pathogens and Disease*, 7(5), 549-554.

Kawasaki, S., Fratamico, P. M., Kamisaki-Horikoshi, N., Okada, Y., Takeshita, K., Sameshima, T., and Kawamoto, S. (2011). Development of the multiplex PCR detection kit for *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157: H7. *Japan Agricultural Research Quarterly: JARQ*, *45*(1), 77-81.

Ke, M., Ramesh, B., Hang, Y., and Liu, Z. (2018). Engineering and characterization of a novel low temperature active and thermo stable esterase from marine *Enterobacter cloacae*. *International Journal of Biological Macromolecules*, 118 (Part A), 304 - 310.

Khalafallah, B. M., El-Tawab, A., Awad, A., Nada, S., and Elkhayat, M. E. (2020). Phenotypic and genotypic characterization of *pseudomonas* species isolated from frozen meat. *Benha Veterinary Medical Journal*, 39(2), 47-51.

Khalifa, E. M. I., and Abd El-Shaheed, Y. T. U. (2005). Bateriological evaluation of chicken meat and some chicken meat products sold in Kafr El-Sheikh. *In 4th International Science Conference* (pp. 135-144).

Kingry, L., and Petersen, J. (2014). Comparative review of *Francisella tularensis* and Francisella novicida. *Frontiers in Cellular and Infection Microbiology*, *4*(35). doi:10.3389/fcimb.2014.00035**.**

Kong, R. Y. C., Lee, S. K. Y., Law, T. W. F., Law, S. H. W., and Wu, R. S. S. (2002). Rapid detection of six types of bacterial pathogens in marine waters by multiplex PCR. *Water research*, 36(11), 2802-2812.

Kunadu, A. P. (2018). The microbiological quality of imported frozen chicken drumsticks from retail meat shops in Accra, Ghana .*Science and Development* ,2, 1,(pp.12-18).

Kupradit, C., Ruamkuson, D., Rodtong, S., and Ketudat-Cairns, M. (2013). Novel multiplex polymerase chain reaction and an oligonucleotide array for specific detection of the dominant foodborne bacterial pathogens in chicken meat. *African Journal of Microbiology Research*, 7(24), 3085-3095.

Laboratório, S. P. (2006). Microbiologia-Nº. de Análises Realizadas por Tipo de Amostras e Parâmetros. *Boletim Estatístico, Serviços de Saúde, Macau SAR; Annu*. Rep, 89.

Ledward, D.A.(2003). "Meat preservation" . Encyclopedia of food Sciences and Nutrition, Academic Press, London, 2nd Ed, pp. 3772-3777, ISBN 9780122270550.

Lee, N., Kwon, K. Y., Oh, S. K., Chang, H. J., Chun, H. S., and Choi, S. W. (2014). A multiplex PCR assay for simultaneous detection *of Escherichia coli* O157: H7, *Bacillus cereus, Vibrio para haemolyticus, Salmonella* spp., *Listeria monocytogenes, and Staphylococcus aureus* in Korean ready-to-eat food. *Foodborne pathogens and disease*, 11(7), 574-580.

Lei, I. F., Roffey, P., Blanchard, C., and Gu, K. (2008). Development of a multiplex PCR method for the detection of six common foodborne pathogens. *Journal of Food and Drug analysis*, 16(4),37-48.

Lianou, A., Panagou, E. Z., and Nychas, G. J. (2016). Microbiological spoilage of foods and beverages. *In The stability and shelf life of food* ,(pp. 3-42). Woodhead Publishing.

Li, J., and Zhang, S. (2020). *Kocuria coralli* sp. nov., a novel *actinobacterium* isolated from coral reef seawater. *International journal of systematic and evolutionary microbiology*, 70(2), 785-789.

Lin, J., Zhao, F., Feng, Y., and Zong, Z. (2017). Draft genome sequence of a high-level colistin-resistant clinical strain of the *Enterobacter cloacae* complex. *Genome announcements*, *5*(14), e00131-17.

Liu, L., Zhu, W., Cao, Z., Xu, B., Wang, G., and Luo, M. (2015). High correlation between genotypes and phenotypes of environmental bacteria *Comamonas testosteroni* strains. *BMC genomics*, *16*(1), 1-14.

Logan, N. A. (2012). *Bacillus* and relatives in foodborne illness. *Journal of applied microbiology*, 112(3), 417-429.

Lund, B., Baird-Parker, A. C., Baird-Parker, T. C., Gould, G. W., and Gould, G. W. (Eds.). (2000). Microbiological safety and quality of food .Springer Science and Business Media. (Vol. 1,p.616).

Lund, B. M., Baird-Parker, T. C., and Gould, G. W. (2000). The microbiological safety and quality of food, Aspen publisher Inc.: New York, NY, USA, Volume 2, p. 1399.

Machado, M. A. A., Ribeiro, W. A., Toledo, V. S., Ramos, G. L. P. A., Vigoder, H. C., and Nascimento, J. S. (2020). Antibiotic resistance and biofilm production in catalase-positive Gram-positive cocci isolated from brazilian pasteurized milk. *Journal of food quality and hazards control*, 7, 67-74.

Madoroba, E., Magwedere, K., Chaora, N. S., Matle, I., Muchadeyi, F., Mathole, M. A., and Pierneef, R. (2021). Microbial communities of meat and meat products: an exploratory analysis of the product quality and safety at selected enterprises in south Africa. *Microorganisms*, *9*(3), 507.

Mansour, A. M. A., Ishlak, A. M. M., and Haj-Saeed, B. A. (2019). Evaluation of bacterial contamination on local and imported mutton in meat markets in Benghazi-Libya. *International Journal of Agricultural Science*, 4,77-83.

Mantilla, S. P. S., Santos, É. B., Vital, H. D. C., Mano, S. B., Freitas, M. Q. D., and Franco, R. M. (2011). Microbiology, sensory evaluation and shelf life of irradiated chicken breast fillets stored in air or vacuum. *Brazilian Archives of Biology and Technology*, 54(3), 569-576.

Mariutti, L. R., and Bragagnolo, N. (2017). Influence of salt on lipid oxidation in meat and seafood products: A review. *Food Research International*, 94, 90– 100.

Martuscelli, M., Serio, A., Capezio, O.,and Mastrocola, D. (2020). Safety, quality and analytical authentication of ḥalāl meat products, with particular emphasis on Salami: A review. *Foods*, 9(8), 1111.

Marx, J. C., Collins, T., D'Amico, S., Feller, G., and Gerday, C. (2007). Cold-adapted enzymes from marine Antarctic microorganisms. *Marine Biotechnology, 9* (3), 293 - 304.

Mikkola, R., Kolari, M., Andersson, M. A., Helin, J., & Salkinoja-Salonen, M. S. (2000). Toxic lactonic lipopeptide from food poisoning isolates of *Bacillus licheniformis*. European journal of biochemistry, 267(13), 4068-4074.

Mishra, A. K., Sharma, K., and Misra, R. S. (2010). Isozyme and PCRbased genotyping of epidemic Phytophthora colocasiae associated with taro leaf blight. *Archives of Phytophathology and Plant Protection*, 43(14): 1367-1380.

Miyoshi, T., Iwatsuki, T., and Naganuma, T. (2005). Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2- micrometer-pore-size filters. *Applied and environmental microbiology*,71(2),1084-1088.

Mohammed, F. A.(2011): The Incidence of enterobacteriaceae fausing Food poisoning in some meat products. *Advanced Journal of Food Science and Technology*, 3(2): 116-121.

Murad, H. O., Al-Jaff, B. M., and Salih, H. H. (2014). Bacteriological quality of imported frozen chicken in Sulaimani markets. *Science Journal of University of Zakho*, 2(1), 124-137.

National Research Council.(2003). Meat and poultry inspection, National Academy of Science,1st ed, PP424.

Nattress, F. M., Yost, C. K., and Baker, L. P. (2001). Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. *International Journal of Food Microbiology*, 70(1–2), 111– 119.

Newkirk, K., Feng, W., Jiang, W., Tejero, R., Emerson, S. D., Inouye, M., and Montelione, G. T. (1994). Solution NMR structure of the major cold shock protein (CspA) from C: identification of a binding epitope for DNA. *Proceedings of the National Academy of Sciences*, *91*(11), 5114-5118.

Noor Uddin, G. M., Larsen, M. H., Guardabassi, L., and Dalsgaard, A. (2013). Bacterial flora and antimicrobial resistance in raw frozen cultured seafood imported to Denmark. *Journal of food protection*, 76(3), 490-499.

Nychas, G. E., and Panagou, E. (2011). Microbiological spoilage of foods and beverages. *In Food and beverage stability and shelf life* (pp. 3-28). Woodhead Publishing**.**

Nychas, G. J. E., Skandamis, P. N., Tassou, C. C., and Koutsoumanis, K. P. (2008). Meat spoilage during distribution. *Meat science*, 78(1–2), 77– 89.

Nyenje, M. E., Tanih, N. F., Green, E., and Ndip, R. N. (2012). Current status of antibiograms of *Listeria ivanovii* and *Enterobacter cloacae* isolated from ready-to-eat foods in Alice, South Africa. *International Journal of Environmental Research and Public Health*, 9(9), 3101-3114.

Octavian, B., Octavian, B., and Aida, V. (2010). Microbial and biogenic amines variation of poultry meat comparison between white and red meat Annals. *Food Science and Technology*, 11, 69-73.

Odeyemi, O. A., Alegbeleye, O. O., Strateva, M., and Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive reviews in food science and food safety*, 19(2), 311-331.

Oranusi, S. U., Obioha, T. U., and Adekeye, B. T. (2014). Investigation on the microbial profile of frozen foods: fish and meat. *International Journal of Advanced Research in Biological Sciences*, 1(2), 71-78.

Otto-Karg, I., Jandl, S., Müller, T., Stirzel, B., Frosch, M., Hebestreit, H., and Abele-Horn, M. (2009). Validation of Vitek 2 nonfermenting Gram-negative cards and Vitek 2 version 4.02 software for identification and antimicrobial susceptibility

testing of nonfermenting gram-negative rods from patients with cystic fibrosis. *Journal of clinical microbiology*, *47*(10), 3283-3288.

Parte, A. C. (2014). LPSN—list of prokaryotic names with standing in nomenclature. *Nucleic acids research*, 42(D1), D613-D616.

Parte, A. C., Carbasse, J. S., Meier-Kolthoff, J. P., Reimer, L. C., and Göker, M. (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *International journal of systematic and evolutionary microbiology*, 70(11), 5607-5612.

Patel, J. B. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. *Molecular diagnosis*, 6(4), 313-321.

Pennacchia, C., Ercolini, D., and Villani, F. (2011). Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food microbiology*, 28(1), 84-93.

Perry, L., Heard, P., Kane, M., Kim, H., Savikhin, S., DomINguez, W., and Applegate, B. (2007). Application of multiplex polymerase chain reaction to the detection of pathogens in food. *Journal of Rapid Methods and Automation in Microbiology*, 15(2), 176-198.

Petruzzi, L., Corbo, M. R., Sinigaglia, M., and Bevilacqua, A. (2017). Microbial spoilage of foods: fundamentals. *In The microbiological quality of food*, Wood head Publishing. (pp. 1-21).

Pincus, D. H. (2006). Microbial identification using the bioMérieux Vitek® 2 system. *Encyclopedia of Rapid Microbiological Methods. Bethesda*, MD: Parenteral Drug Association, 1-32.

Plavsic, D., Okanovic, D., Gubic, J., and Njezic, Z. (2015). Microbiological and chemical evaluation of dried smoked meat product. *Procedia Food Science*, 5, 239– 242.

Provincial, L., Guillén, E., Alonso, V., Gil, M., Roncalés, P., and Beltrán, J. A. (2013). Survival of *Vibrio parahaemolyticus* and *Aeromonas hydrophila* in sea

bream (Sparus aurata) fillets packaged under enriched CO2 modified atmospheres. *International Journal of Food Microbiol*. 166, 141–147.

Rahman, M. S., and Velez-Ruiz, J. F. (2007). Food preservation by freezing. *In Handbook of food preservation* (pp. 653-684). CRC Press.

Rajendran, C., Mahesh, S., and Jayathilakan, K. (2019). Advances in meat preservation and safety. *International Journal of Science Res*earch, 10, 1499-1502.

Rapuntean, G., and Rapuntean, S. (2005). Bacteriologie veterinara speciala, Ed. *Academic Pres, Cluj-Napoca*,p.136.

Rawat, S. (2015). Food spoilage: microorganisms and their prevention. *Asian Journal of Plant Science and Research*, 5(4), 47-56.

Rey, M. W., Ramaiya, P., Nelson, B. A., Brody-Karpin, S. D., Zaretsky, E. J., Tang, M., ... and Berka, R. M. (2004). Complete genome sequence of the industrial bacterium *Bacillus licheniformis* and comparisons with closely related Bacillus species. *Genome biology*, 5(10), 1-12.

Rodpai, E.; Moongkarndia,P.; Tungrugsasutb , W.; Phisannoradeja , R., and Kanarat, S.(2013). Comparison of multiplex polymerase chain reacti reaction and immunoassay to detect *Salmonella* spp., *S. T yphimurium,* and *S. Enteritidis* in Thai chicken meat. *Science Asia* ,39 : 150–159.

Rossi, C., Serio, A., Chaves-López, C., Anniballi, F., Auricchio, B., Goffredo, E., ... and Paparella, A. (2018). Biofilm formation, pigment production and motility in *Pseudomonas* spp. isolated from the dairy industry. *Food Control*, *86*, 241-248.

Roth, T. M. (2016). The ecology and epidemiology of *Francisella tularensis* in the California central valley. University of California, Davis.

Rudolph, W. W., Gunzer, F., Trauth, M., Bunk, B., Bigge, R., and Schröttner, P. (2019). Comparison of VITEK 2, MALDI-TOF MS, 16S rRNA gene sequencing and whole-genome sequencing for identification of *Roseomonas mucosa*. *Microbial pathogenesis*, 134, 103576.

Russo, F., Ercolini, D., Mauriello, G., and Villani, F. (2006). Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. *Food Microbiology*, 23(8), 797– 802.

Saad, M. S., Hassan, M. A., and Elgnainy, N. F. (2018). Evaluation of the quality of the minced meat in Egyptian markets. *Benha Veterinary Medical Journal*, 35(2), 257-268.

Säde, E., Penttinen, K., Björkroth, J., and Hultman, J. (2017). Exploring lot-to-lot variation in spoilage bacterial communities on commercial modified atmosphere packaged beef. *Food Microbioogy* ,62, 147–152.

Sadeq, J. N., Mohamed, B. J., and Hussain, M. H. (2018). Isolation and identification of some bacteria from imported meat (beef burger) by using vitek2 technique. A*l-Qadisiyah Journal of Veterinary Medicine Sciences*, 17(2), 117-124.

Sadiku, M. N., Ashaolu, T. J., and Musa, S. M. (2020). Food contamination: a primer. *International Journal of Advances in Scientific Research & Engineering*, 6(3), 1-7.

Saeed ,A.A.; Hasoon ,M.F., and Mohammed,M.H.(2013).Isolation and molecular identification of *Salmonella typhimurium* from chicken meat in iraq. *Journal of World's Poultry Research*, 3(2): 63-67.

Salkinoja-Salonen, M.S., Vuorio, R., Andersson, M.A., Ka¨mpfer, P., Andersson, M.C., Honkanen-Buzalski, T. and Scoging, A.C. (1999) Toxigenic strains of *Bacillus licheniformis* related to food poisoning. *Applied and Environmental Microbiol*ogy, 65(10), 4637–4645.

Sambrook, J., and Russell, D. W. (2006). Detection of DNA in agarose gels. *Cold Spring Harbor Protocols*, 2006(1), pdb-prot4022

Saraiva, C., Fontes, M. D. C., Patarata, L., Martins, C., Cadavez, V., and Gonzales-Barron, U. (2016). Modelling the fate of *Listeria monocytogenes* in beef meat stored at refrigeration temperatures under different packaging conditions. *Procedia Food Science*, 7, 177–180.

Savini, V., Catavitello, C., Masciarelli, G., Astolfi, D., Balbinot, A., Bianco, A., ... and D'Antonio, D. (2010). Epidemiology, pathogenicity and drug resistance of members of the genus *Kocuria*. *Journal of Medical Microbiology*, 59(12), 1395-1402.

Sayed, H. K., Tolba, K. S., Sobhy, H. M., and Hekal, S. H. A. (2021). Assuring the safety of local and imported beef meat from different slaughterhouses in Egypt. *Advances in Animal and Veterinary Sciences*, 9(9), 1472-1482.

Schuerger, A. C., Ulrich, R., Berry, B. J., and Nicholson, W. L. (2013). Growth of *Serratia liquefaciens* under 7 mbar, 0 C, and CO2-enriched anoxic atmospheres. *Astrobiology*, 13(2), 115-131.

Schuerger, A. C., and Nicholson, W. L. (2016). RETRACTED: Twenty-three species of hypobarophilic bacteria recovered from diverse ecosystems exhibit growth under simulated martian conditions at 0.7 kPa. *Astrobiology*, 16(5), 335-347.

Shaltout, F. A., Maarouf, A. A., and Mohamed, H. A. (2017). Bacteriological aspect of frozen beef burger. *Ecronicon Nutrition*, 10(4), 162-172.

Sheir, S. H., Ibrahim, H. M., Hassan, M. A., and Shawky, N. A. (2020). Incidance of psychotropic bacteria in frozen chicken meat products with special reference to *Pseudomonas* species. *Benha Veterinary Medical Journal*, 39(1), 165-168.

Shon, A. S., Bajwa, R. P., and Russo, T. A. (2013). Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. *Virulence*, 4(2), 107-118.

Skurnik, D., Ruimy, R., Andremont, A., Amorin, C., Rouquet, P., Picard, B., and Denamur, E. (2006). Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*. *Journal of Antimicrobial chemotherapy*, *57*(6), 1215-1219.

Snyder, A. B., and Worobo, R. W. (2018). The incidence and impact of microbial spoilage in the production of fruit and vegetable juicesas reported by juice manufacturers. *Food Control*, 85, 144–150.

Sohaib, M., Anjum, F. M., Arshad, M. S., and Rahman, U. U. (2016). Postharvest intervention technologies for safety enhancement of meat and meat based products; a critical review. *Journal of food science and technology*, *53*(1), 19-30.

Spanu, C., Piras, F., Mocci, A. M., Nieddu, G., De Santis, E. P. L., and Scarano, C. (2018). Use of *Carnobacterium* spp protective culture in MAP packed Ricotta fresca cheese to control *Pseudomonas spp*. *Food microbiology*, 74, 50-56.

Srey, S., Jahid, I. K., and Ha, S. D. (2013). Biofilm formation in food industries: a food safety concern. *Food control*, 31(2), 572-585.

Stephen, J.F., (2020).“Food preservation and spoilage organisms”, The Microbiology of Safe Food, 3rd Ed, pp. 85-128.

Stratev, D., Vashin, I., and Daskalov, H. (2015). Microbiological status of fish products on retail markets in the Republic of Bulgaria. *International Food Research Journal*, 22(1),64-69.

Suardana, I. W. (2014). Analysis of nucleotide sequences of the 16S rRNA gene of novel *Escherichia coli* strains isolated from feces of human and Bali cattle. *Journal of nucleic acids*, 2014,p7.

Sultana, F., Afroz, H., Jahan, A., Fakruddin, M., and Datta, S. (2014). Multi–antibiotic resistant bacteria in frozen food (ready to cook food) of animal origin sold in Dhaka, Bangladesh. *Asian Pacific journal of tropical biomedicine*, 4(1), S268-S271.

Sun, R. R. (2015). Detection of foodborne pathogen. *Food Safety Guide,* **4**, 73.

Suo, B., He, Y., Tu, S.I. and Shi, X. (2010) A multiplex realtime polymerase chain reaction for simultaneous detection of *Salmonella* spp., *Escherichia coli* O157, and *Listeria monocytogenes* in meat products. *Foodborne Pathogen and Disease*, 7, 619– 628.

Switaj, T. L., Winter, K. J., and Christensen, S. (2015). Diagnosis and management of foodborne illness. *American family physician*, *92*(5), 358-365.

Tao, J., Liu, W., Ding, W., Han, R., Shen, Q., Xia, Y., ... and Sun, W. (2020). A multiplex PCR assay with a common primer for the detection of eleven foodborne pathogens. *Journal of food science*, 85(3), 744-754.

Tavakol, M., Momtaz, H., Mohajeri, P., Shokoohizadeh, L., and Tajbakhsh, E. (2018). Genotyping and distribution of putative virulence factors and antibiotic resistance genes of *Acinetobacter baumannii* strains isolated from raw meat. *Antimicrobial Resistance & Infection Control*, *7*(1), 1-11.

Uzair, B., Menaa, F., Khan, B. A., Mohammad, F. V., Ahmad, V. U., Djeribi, R., and Menaa, B. (2018). Isolation, purification, structural elucidation and antimicrobial activities of kocumarin, a novel antibiotic isolated from *actinobacterium Kocuria* marina CMG S2 associated with the brown seaweed Pelvetia canaliculata. *Microbiological research*, 206, 186-197.

Velusamy, V.; Arshak, K.;Korostynsak,O.;Oliwa, K., and adley ,C.(2010).An overview of food-borne pathogen detection: in the perspective of biosensors. *Biotechnology Advanced*,28(2):232- 254.

Větrovský, T., and Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PloS one*, 8(2), e57923.

Wang, G. Y., Wang, H. H., Han, Y. W., Xing, T., Ye, K. P., Xu, X. L., and Zhou, G. H. (2017). Evaluation of the spoilage potential of bacteria isolated from chilled chicken *in vitro* and *in situ*. *Food Microbiology*, 63, 139– 146.

Wang, L., Li, Y. and Mustaphai, A. (2007) Rapid and simultaneous quantitation of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* in ground beef by multiplex real-time PCR and immunomagnetic separation. *Journal of Food Protection*, 70, 1366–1372.

Wei, Q., Wang, X., Sun, D. W., and Pu, H. (2019). Rapid detection and control of psychrotrophic microorganisms in cold storage foods: A review. *Trends in Food Science & Technology*, 86, 453-464.

Wirth, S. E., Ayala-del-Río, H. L., Cole, J. A., Kohlerschmidt, D. J., Musser, K. A., Sepúlveda-Torres, L. D. C., ... and Wolfgang, W. J. (2012). Psychrobacter sanguinis sp. nov., recovered from four clinical specimens over a 4-year period. *International journal of systematic and evolutionary microbiology*, *62*(1), 49-54.

Woo, P. C. Y., Leung, P. K. L., Leung, K. W., and Yuen, K. Y. (2000). Identification by 16S ribosomal RNA gene sequencing of an enterobacteriaceae species from a bone marrow transplant recipient. *Molecular pathology*, 53(4), 211-215.

WHO: World Health Organization (2020). Clinical consortium on healthy ageing 2019: report of consortium meeting held 21-22 November 2019, Geneva, Switzerland.

WHO: World Health Organization (2015). Estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization, Geneva, Switzerland.

Wu, B., Tong, X., He, H., Yang, Y., Chen, H., Yang, X., and Xu, B. (2021). Misidentification of *Burkholderia pseudomallei*, China. *Emerging infectious diseases*, 27(3), 964-966.

Yang, Q., Zhao, S., Kučerová, Z., Stejskal, V., Opit, G., Qin, M., ... and Li, Z. (2013). Validation of the 16S rDNA and COI DNA barcoding technique for rapid molecular identification of stored product psocids (Insecta: Psocodea: Liposcelididae). *Journal of Economic Entomology*, 106(1), 419-425.

Yang, S. C., Lin, C. H., Aljuffali, I. A., and Fang, J. Y. (2017). Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. *Archives of microbiology*, 199(6), 811-825.

Yar, D. D., Kwenin, W. K., Zanu, W. K., Balali, G. I., Adepa, E. K., and Gyapong, F. (2020). Microbial quality of frozen chicken parts from three import countries into the Kumasi Metropolis of Ghana. *Preprints*,v1 ,2020120254.

Yassin, S. A., Nadhom, B. N., and Al-Gburi, N. M. (2021). Detection of *Listeria monocytogenes* in several types of frozen meat in Baghdad city. *Iraqi Journal of Science*,62(3), 742-750

Younes, O., Ibrahim, H., Hassan, M., and Amin, R. (2019). Demonstration of some food borne pathogens in different meat products: a comparison between conventional and innovative methods. *Benha Veterinary Medical Journal*, 36(2), 219-228.

Zahid, S., Fleming, T., and Randall, G. K. (2010). Microbial growth in ground beef during different methods of thawing. Undergraduate Research Journal for the Human Sciences, 9(1).

Zarei ,M.; Basiri ,N.; Jamnejad ,A. , and Hadi Eskandari , M . (2013). Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. in Beef, Buffalo and Lamb using multiplex PCR. *Jundishapur Journal Microbiology* , 6 (8) : 7244-7248.

Zhang, D. X., Kang, Y. H., Song, M. F., Shu, H. P., Guo, S. N., Jia, J. P., ... and Shan, X. F. (2019). Identity and virulence properties of *Aeromonas* isolates from healthy Northern snakehead (Channa argus) in China. *Letters in applied microbiology*, 69(2), 100-109.

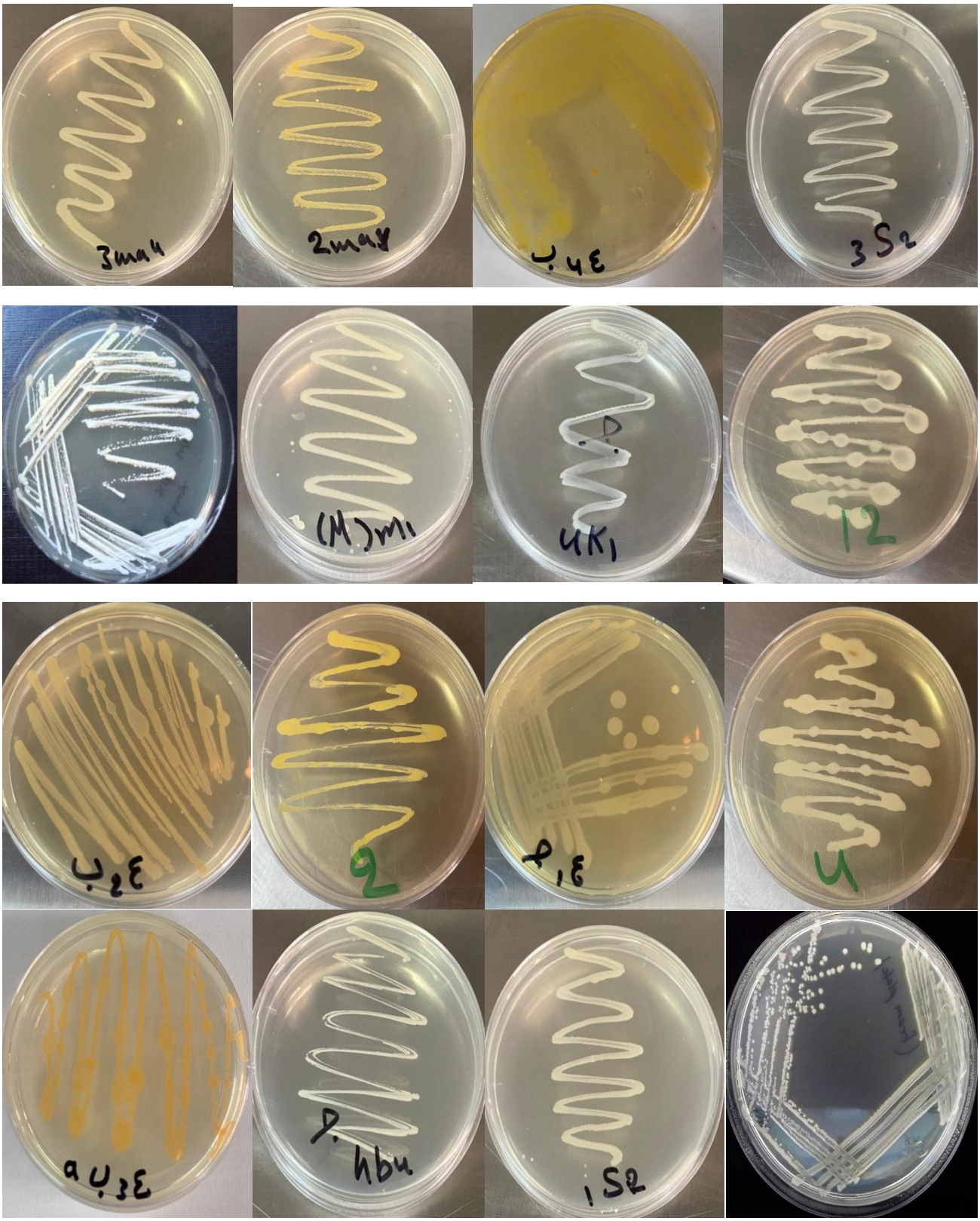
Zhang, Y., Li, D., Lv, J., Li, Q., Kong, C., and Luo, Y. (2017). Effect of cinnamon essential oil on bacterial diversity and shelf-life in vacuum-packaged common carp (Cyprinus carpio) during refrigerated storage. *International Journal of Food Microbiology*, 249, 1-8.

Zhao ,H.K1.; Wang ,W.J.; Zhao, P.; Zhang ,H.B.; Kong, N. and Zhou, M.D. (2014). Simultaneous detection of *Salmonella, Listeria Monocytogenes* and *Shigella* in Poultry Samples by Triplex PCR. *Journal of Bacteriology and Mycology*, 1(1), 1-5.

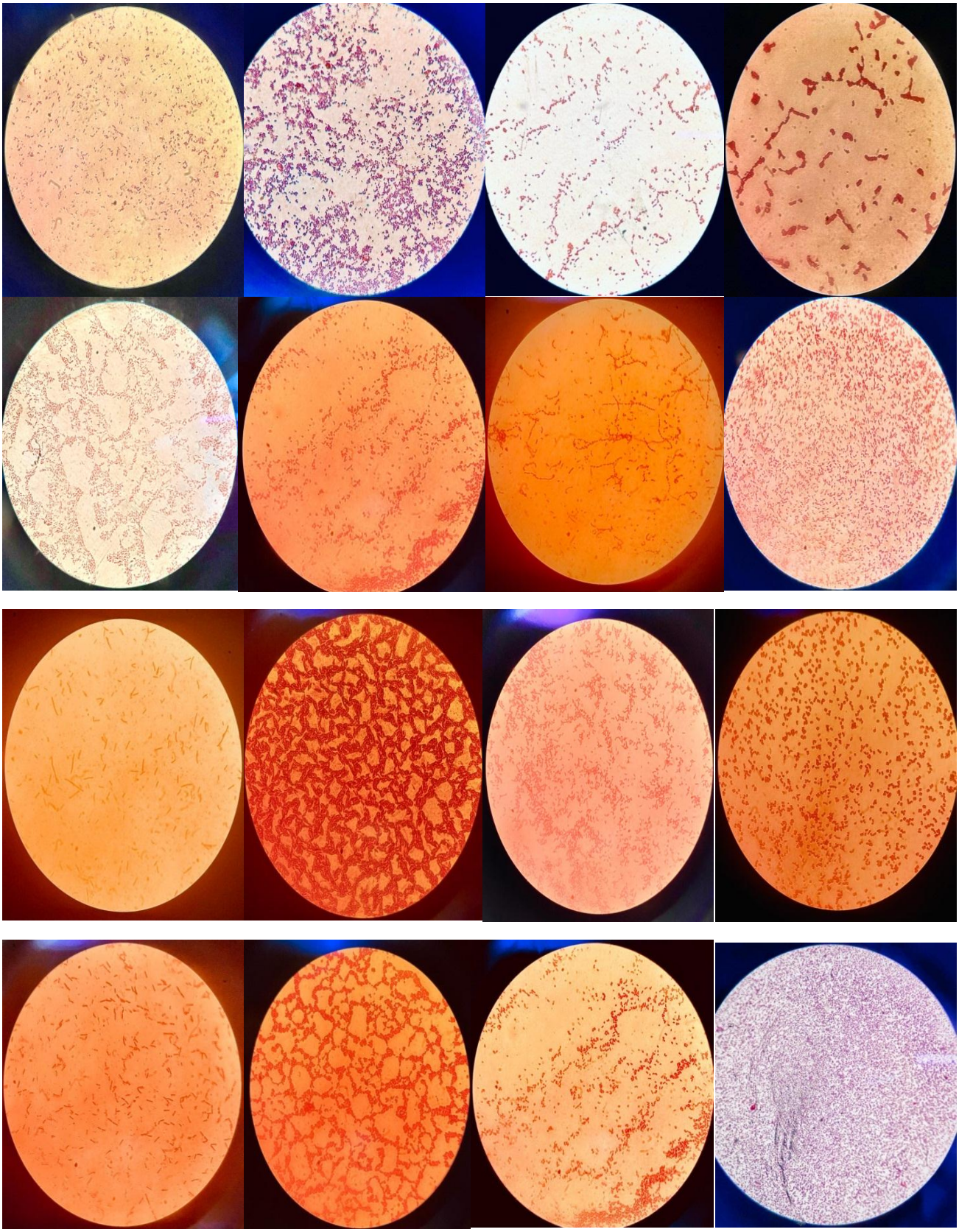
Zhao, X., Lin, C. W., Wang, J., and Oh, D. H. (2014). Advances in rapid detection methods for foodborne pathogens. *Journal of microbiology and biotechnology*, 24(3), 297-312.

APPENDICES

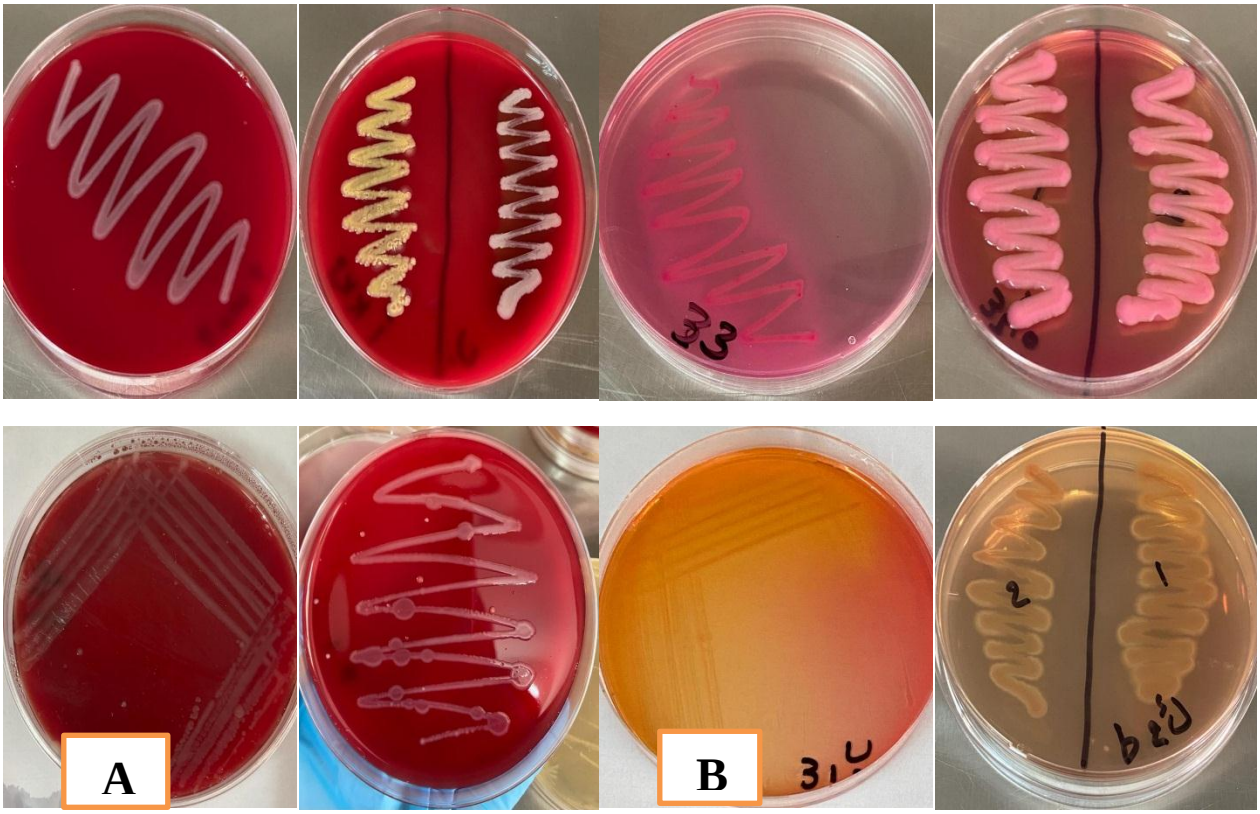
Appendix (1): A pure culture of isolated bacteria.



Appendix (2): Gram stain of bacterial isolates, examined under light microscope with magnification power 1000x.



Appendix (3) : (A) The bacterial growth on the Blood agar. (B) The bacterial growth on the MacConkey agar.



Appendix (4): Biochemical tests of bacterial isolates by Vitek-2 system:

Isolates numbers	Bionumber	probability
M1	0000003100000040	99%
M2	0000001000000000	97%
M3	6607734753565010	99%
M4	4040000000000000	92%
M5	1003001100000040	95%
M6	0067213140000001	93%
M7	0001011103500352	97%

M8	0003011103500210	99%
M9	4427515555556230	99%
M10	5001611153500210	91%
M11	0600010000000201	Misidentified
M12	040000200000201	88%
M13	100012300101030	86%
M14	150006463773671	87%
M15	414030300431201	95%
M16	000010100000000	99%
M17	060003015770331	88%
M18	2301011100000000	Misidentified
M19	010010102000000	98%
M20	010004000660031	95%
M21	010000076720231	92%
M22	420046015671331	91%
M23	104000010000002	94%
M24	2727635773553010	86%
M25	000010100040000	99%
P1	502000445767531	90%
P2	2301614474000210	87%
P3	041030100000030	Misidentified
P4	070101201362531	87%
P5	4221510450240210	98%
P6	000000004320211	96%
P7	0605613440000200	86%
P8	000004000020021	93%
P9	5620704150260000	99%
P10	0000000301501001	99%

P11	2301714474000000	99%
P12	4003100100200000	97%
P13	010000001463431	97%
P14	2701614570000000	99%
P15	010012342220030	Misidentified
P16	010002021720221	Misidentified

Organism Quantity:
Selected Organism : Pseudomonas alcaligenes

Comments:	

Identification Information	Analysis Time:	9.25 hours	Status:	Final
Selected Organism	99% Probability Bionumber:	Pseudomonas alcaligenes 0000003100000040		
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	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	lMLTa	+	62	ELLM	-	64	lLATa	-			

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Comments:	

Identification Information	Analysis Time:	4.00 hours	Status:	Final
Selected Organism	99% Probability Bionumber:	Klebsiella pneumoniae ssp pneumoniae 6607734753565010		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	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	-			

Organism Quantity:
Selected Organism : Acinetobacter baumannii complex

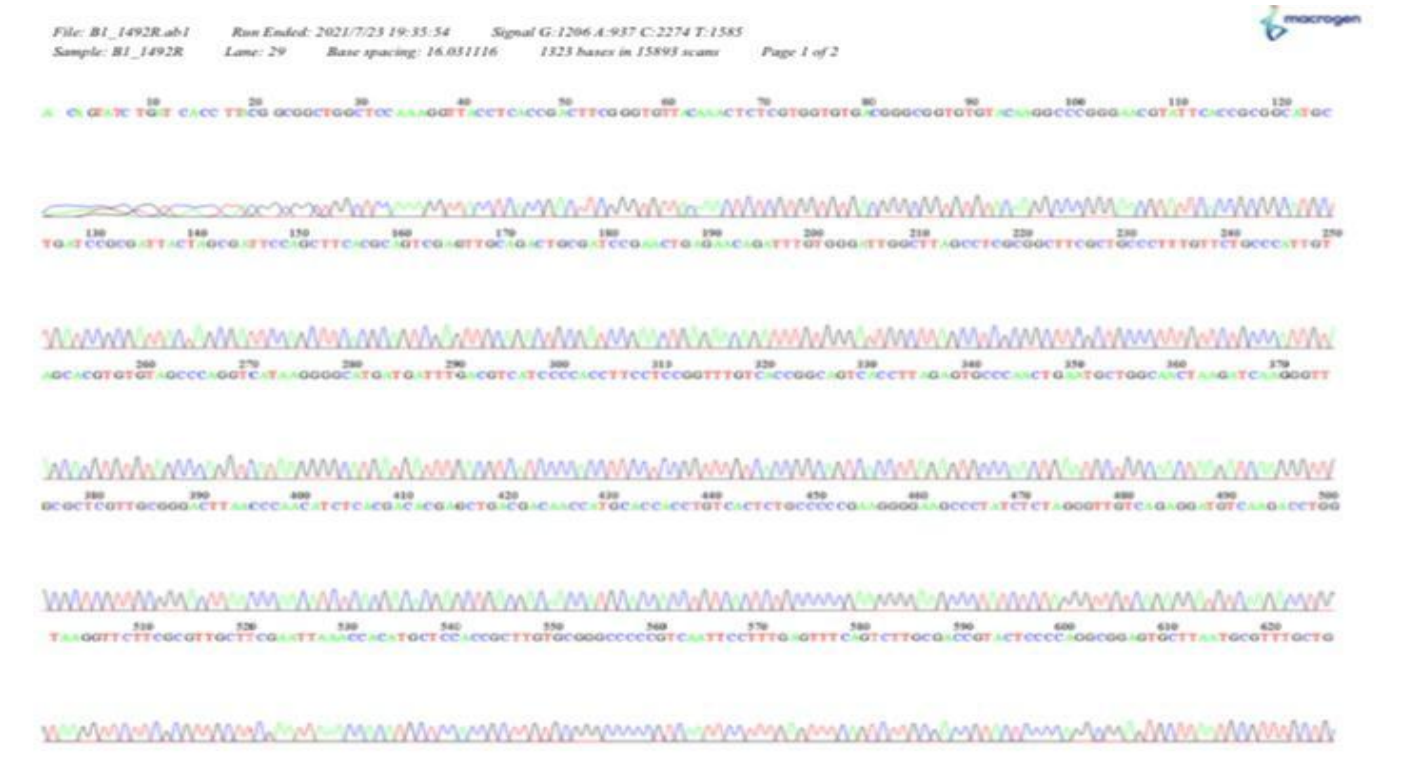
Comments:	

Identification Information	Analysis Time:	8.75 hours	Status:	Final
Selected Organism	97% Probability Bionumber:	Acinetobacter baumannii complex 0001011103500352		
ID Analysis Messages				

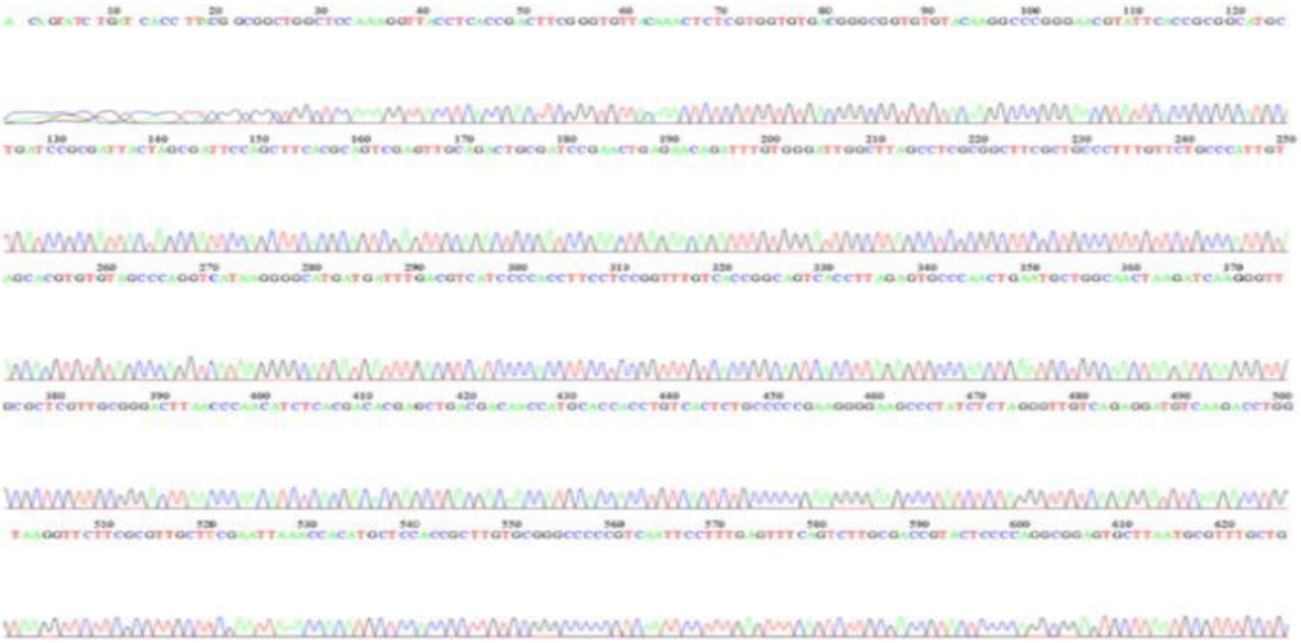
Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	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	+			

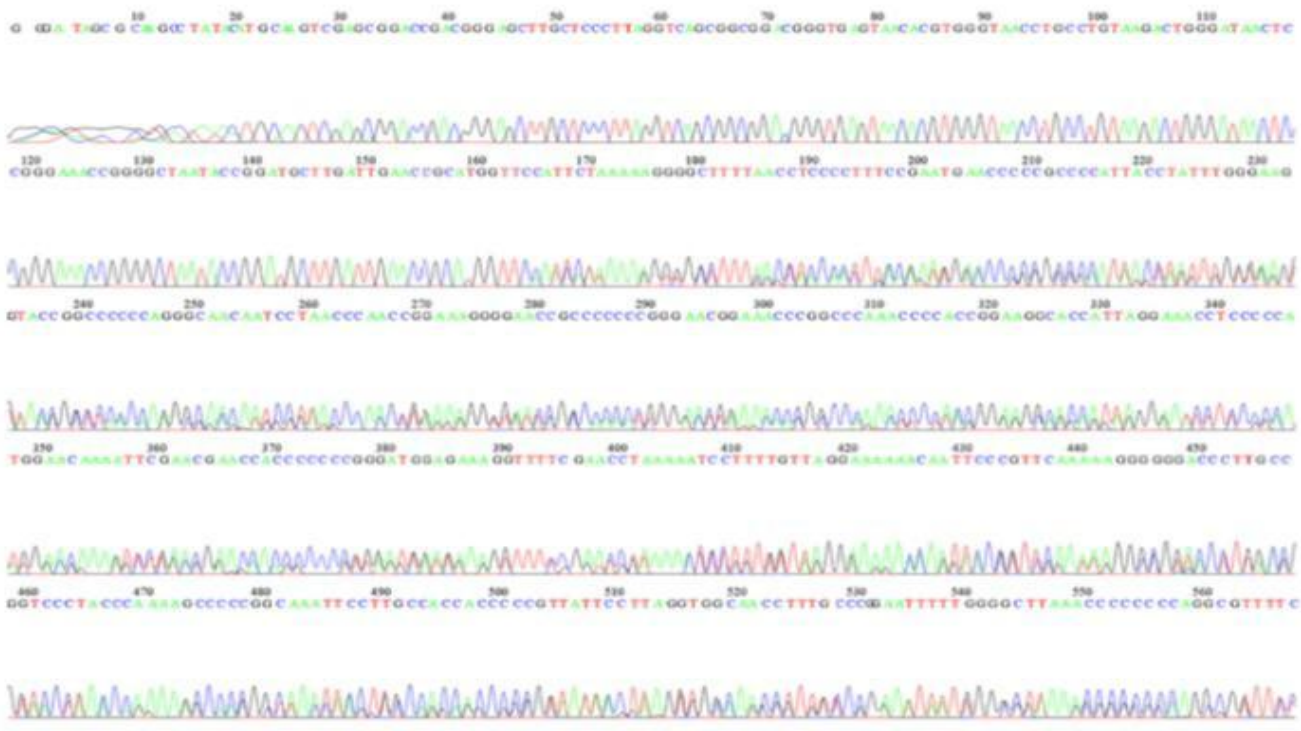
Appendix (5) : DNA sequencing :-



File: B1_1492R.ab1 Run Ended: 2021/7/23 19:35:54 Signal G:1206 A:937 C:2274 T:1585
Sample: B1_1492R Lane: 29 Base spacing: 16.031116 1325 bases in 15893 scans Page 1 of 2



File: B1_27F.ab1 Run Ended: 2021/7/23 19:35:54 Signal G:1627 A:2020 C:3342 T:2181
Sample: B1_27F Lane: 31 Base spacing: 16.006386 1572 bases in 21177 scans Page 1 of 2



الخلاصة

أجريت الدراسة الحالية لغرض عزل وتوصيف بعض انواع البكتريا من الاطعمة المجمدة بالطرق البايوكيميائية والجزيئية . خلال فترة ستة اشهر بين شهر تشرين الثاني 2020 وشهر نيسان 2021، تم جمع 100 عينة من الاغذية المجمدة وبشكل عشوائي من الاسواق التجارية في محافظة ميسان وثم تم نقلها في ظروف معقمه الى مختبر الاحياء المجهرية في كلية العلوم . شملت عينات الأغذية المجمدة: منتجات لحوم من شركات محلية ومستوردة متمثلة في (برجر – صوصج - كباب - شاورما - لحوم مفرومة) ومنتجات الدجاج من الشركات المحلية والمستوردة متمثلة في (صدر - فخذ - كبدة - برجر - كباب). حيث يتم أخذ خمس عينات لكل منتج مستخدم.

أظهرت نتائج منتجات اللحوم المجمدة في الدراسة الحالية ان المتوسط الحسابي لعينات البرجر والصوصج والشاورما المستوردة هو $10^{13}\times1.44\pm{}^{b}10^{13}\times2.52$ ، $10^{13}\times2.92\pm{}^{b}10^{13}\times3.86$ و $10^{13}\times2.78$ $10^{13}\times1.12\pm{}^{a}$ على التوالي بينما كان المتوسط الحسابي للعينات المحلية لهذه المنتجات كالتالي $10^{13}\times2.32$ $10^{13}\times1.06\pm$ ، $10^{13}\times0.16\pm{}^{b}10^{13}\times2.85$ و $10^{13}\times2.18\pm{}^{a}10^{13}\times2.99$ على التوالي لذا فإن العينات المستوردة من هذه الأنواع الثلاثة من منتجات اللحوم كانت لها أعلى قيمة من العد الكلي الهوائي(Aerobic Plate Count) من العينات المحلية ،اما اللحم المفروم ومنتج الكباب فقد تم جمعه من شركتين محليتين مختلفتين رمز لهما ب (L1,L2) فظهرت عينات اللحوم المفرومة من L1 بمتوسط حسابي قيمته $10^{12}\times3.10\pm{}^{a}10^{12}\times5.56$ اما عينات L2 فكان المتوسط الحسابي لها $10^{13}\times0.4\pm{}^{a}10^{13}\times2.59$ لذا فإن العينات من الشركة L1 تحتوي على قيم عالية من العد الكلي الهوائي من عينات الشركة L2 ،واخيرا ظهرت عينات الكباب من الشركة L1 بمتوسط حسابي هو $10^{13}\times1.81\pm{}^{b}10^{13}\times2.46$ بينما عينات الكباب للشركة L2 كان المتوسط الحسابي لها هو $10^{13}\times0.19\pm{}^{b}10^{13}\times1.24$ لذلك فإن عينات الكباب من L1 تحتوي على نسبة اعلى من حيث العد الكلي الهوائي من عينات L2.

أظهرت نتائج قطع ومنتجات لحوم الدواجن المجمدة أن المتوسط الحسابي لعينات الكبد والصدر والفخذ و البرغر المستوردة $10^{13}\times1.22\pm{}^{b}10^{13}\times1.89$ ، $10^{13}\times2.16\pm{}^{b}10^{13}\times2.91$ ، $10^{13}\times2.78$ $10^{13}\times1.26\pm$ و $10^{13}\times2.07\pm{}^{b}10^{13}\times2.59$ على التوالي اما العينات المحلية لها فكان المتوسط الحسابي لها كالتالي $10^{12}\times4.7\pm{}^{b}10^{12}\times9.2$ ، $10^{13}\times1.19\pm{}^{b}10^{13}\times1.83$ ، $10^{13}\times0.13\pm{}^{b}10^{13}\times1.64$ $10^{13}\times1.29\pm{}^{b}10^{13}\times1.66$ على التوالي لذلك كانت العينات المستوردة ذات قيمة للعد الكلي الهوائي اعلى من العينات المحلية ، تم جمع كباب الدجاج المستخدم في هذه الدراسة من شركتين محليتين هما (L1، L2) فكان المتوسط الحسابي لعينات الشركة هو $10^{13}\times1.23\pm{}^{a}10^{13}\times2.09$ L1 اما عينات الشركة L2 فكان المتوسط

الحسابي لها هو 2.23×10^{13} ª $\pm1.49\times10^{13}$ لذا فإن العد الكلي الهوائي لعينات الكباب من الشركة L2 أعلى من العد الكلي الهوائي لعينات الشركة L1 .

تم عزل العديد من السلالات البكتيرية من الأطعمة المجمدة (اللحوم ومنتجات الدواجن). حيث تم تمييز واحد وأربعين عزلة بكتيرية عن طريق الاختبارات البكتريولوجية العادية والقياسية ، وقد أظهرت صبغة جرام أن البكتيريا السالبة جرام مثلت معظم السلالات البكتيرية في منتج اللحوم (52٪) بينما مثلت البكتريا موجبة الجرام (48٪).اما في عينات الدواجن أظهرت صبغة الجرام أن البكتيريا موجبة الجرام كانت تمثل معظم السلالات البكتيرية (59٪) بينما مثلت (41٪) النسبة المئوية للبكتيريا سالبة جرام . فقط خمسة سلالات بكتيرية لم يتم التعرف عليها باستخدام نظام Vitek2 وتم التعرف عليها باستخدام تقنية Monoplex PCR .تم تشخيص البكتيريا المعزولة بواسطة نظام Vitek-2 والتحليل الجزيئي.

تمثلت الدراسة الجزيئية باستخدام Multiplex PCR و Monoplex PCR حيث استخدمت تقنية Multiplex PCR لتشخيص وجود أنواع شائعة من البكتيريا في عينات الطعام بواسطة بادئات خاصة ، وأعطت نتائج سلبية وكانت هذه النتيجة مطابقه للاختبارات الاولية وكذلك نظام Vitek2 والتي اكدت عدم احتواء العينات على هذه الانواع ، بينما تم استخدام Monoplex PCR لتشخيص ثمانية عشر نوعًا من البكتيريا المعزولة باستخدام بادئ عام وتم دراسة تسلسلات النوكليوتيدات 16S r RNA في المركز الوطني لمعلومات التكنولوجيا الحيوية (NCBI) باستخدام أداة البحث عن المحاذاة المحلية الأساسية (Nucleotide BLAST) .وكانت البكتريا المشخصة كالاتي : *Aeromonas veronii, Pseudomonas plecoglossicida, Acinetobacter lwofii, Aeromonas veronii, Klebsiella pneumoniae, Pseudomonas japonica, Pseudomonas songnenensis, Klebsiella pneumoniae* subsp.ozaenae, *Psychrobacter sagnuinis, Klebsiella pneumoniae, Acinetobacter lwofii, Lysinibacillus boronitolerans, Bacillus licheniformis, Enterobacter hormaechei, Pseudomonas putida, Serratia liquefaciens, Comamonas testosteroni ,and Methylogaea oryzae.*

تم تسجيل ستة عشر عزله بكتيرية في بنك الجينات وتحت اعداد انضمام مختلفة وهي : MZ934693.1 MZ934671.1, MZ931306.1, MZ931286.1, MZ930472.1, MZ927456.1, MZ927229.1, MZ923509.1, MZ923503.1, MZ921931.1, MZ920155.1, MZ919358.1, MZ919316.1, .MZ919316.1, MZ913024.1, MZ911849.1

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

دراسة البكتريا الملوثة للأطعمة المجمدة في المحلات التجارية لمحافظة ميسان/ جنوب العراق

رسالة مقدمة الى

مجلس كلية العلوم / جامعة ميسان وهي جزء من

متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل

دعاء علي حسين

بكالوريوس تقنيات احيائية / جامعة بغداد

(2008)

بأشراف

أ.د. زاهد سعدون عزيز

رجب 1443 هـ **شباط 2022 م**