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Faculty of Graduate Studies

**Polymerase chain reaction for detection of food
borne bacterial pathogens in meat products in
Jenin district-Palestine**

By

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**This Thesis is Submitted in Partial Fulfillment of the requirements for
the Degree of Master in Biological Sciences, Faculty of Graduate
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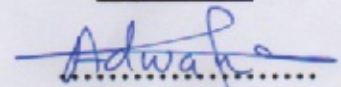
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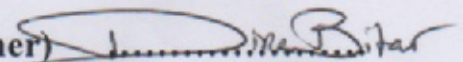
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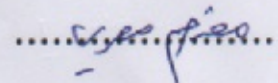
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Dedication

To all of my family, my mother, my father, my husband and my sons, to the souls of the martyrs and my precious nation.

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الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

Polymerase chain reaction for detection of food borne bacterial pathogens in meat products in Jenin district- Palestine

أقر بأن ما شملت عليه هذه الرسالة إنّما هو نتاج جهدي الخاص، باستثناء ما تمّت الإشارة إليه حيثما ورد، وأنّ هذه الرسالة ككل، أو أيّ جزء منها لم يقدّم من قبل لنيل أيّ درجة أو لقب علميّ لدى أيّ مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researchers own work, and has not been submitted elsewhere for any other degree or qualification.

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Date:

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List of Contents

No	Subject	Page
	Dedication	iii
	Acknowledgement	iv
	List of contents	vi
	List of tables	viii
	List of figures	ix
	List of abbreviations	xi
	Abstract	xiii
	Chapter One: Introduction	1
1.1	General background	1
	Chapter Two: Literature Review	5
2.1	Overview of food-borne illness	5
2.1.1	<i>Salmonella</i>	9
2.1.2	<i>Escherichia coli</i>	9
2.1.3	<i>Staphylococcus aureus</i>	10
2.2	Molecular analysis methods	14
2.3	Aims of the study	15
	Chapter Three: Materials and Methods	17
3.1	Sample collection	17
3.2	Media preparation	18
3.2.1	Tryptone Soya Broth-Yeast Extract (TSBYE) media	18
3.2.2	Xylose-Lysine Deoxycholate (XLD) Agar	18
3.2.3	MacConkey Agar	18
3.2.4	Mannitol Salt Agar (MSA)	19
3.3	Food sample preparation bacterial culturing	19
3.4	DNA extraction	20
3.5	Detection of food pathogens by PCR	20
3.5.1	Detection of <i>E. coli</i> <i>Mdh</i> gene	20
3.5.2	Detection of <i>femA</i> (<i>S. aureus</i>) and 1.8-kb <i>HindIII</i> DNA fragment(<i>Salmonella spp</i>)	21
3.5.3	Detection of staphylococcal enterotoxin (<i>sea-see</i>) genes	22
3.5.4	Detection of <i>E. coli</i> pathotypes	22
	Chapter Four: Results	25
4.1	Bacterial enumeration and cultural characterization	25

4.2	Detection of <i>E. coli</i> by PCR	26
4.3	Detection of <i>S. spp</i> and <i>S. aureus</i> by PCR	27
4.4	Detection of staphylococcal enterotoxin (<i>sea-see</i>) genes	28
4.5	Detection of <i>E. coli</i> pathotypes	29
	Chapter Five: Discussion	31
	References	37
	الملخص	ب

List of Tables

No.	Table Title	Page
1.	Some pathogenic microorganisms responsible for foodborne illness (Velusamy et al., 2010).	11
2.	Selected characteristics of pathogenic <i>E. coli</i> groups.	13
3.	Target genes for PCR amplification, amplicon size, primer sequences and annealing temperature.	24
4.	Prevalence of <i>E. coli</i> pathotypes coinfection in 3 types of meat samples.	30

List of Figures

No.	Figure Title	Page
1.	Distribution of samples collection localities in Jenin governorate.	17
2.	Multiplex PCR profiles	27

List of Diagrams

No.	Diagram Title	Page
1.	Morphological characterization on different selective media; MacConkey agar, XLD Agar and MSA.	25
2.	Prevalence of <i>E. coli</i> in beef, chicken and turkey meat.	26
3.	Distribution of <i>Salmonella</i> and <i>S. aureus</i> in three types of meat products using PCR.	28
4.	Distribution of staphylococcal enterotoxin genes (<i>sea-see</i>) in meat samples.	29
5.	Prevalence of <i>E. coli</i> pathotypes EHEC, EPEC, EAEC, DAEC and ETEC with uni-infection in 3 types of meat samples .	30

List of Abbreviations

DNA: Deoxyribonucleic acid

E.coli: *Escherichia coli*

PCR: Polymerase chain reaction

dNTPs: Deoxyribonucleotide triphosphates

EDTA: Ethelendiaminetetraacetic acid

Taq DNA polymerase: *Thermus aquaticus* DNA polymerase

H₂S:Hydrogen Sulfide

MgCl₂: Magnesium chloride

afa: a fimbrial adhesins

EHEC: Enterohemorrhagic *E. coli*

EPEC: Enteropathogenic *E. coli*

EAEC: Enteroaggregative *E. coli*

ETEC: Enterotoxigenic *E. coli*

DAEC: Diffusely adherent *E. coli*

EIEC : Enteroinvasive *E. coli*

HUS: Hemolytic Uremic Syndrome

S. aureus: *Staphylococcus aureus*

SEs :Staphylococcal enterotoxins

SEa : Staphylococcal enterotoxin a

SEb : Staphylococcal enterotoxin b

SEc : Staphylococcal enterotoxin c

SEd : Staphylococcal enterotoxin d

SEe : Staphylococcal enterotoxin e

LEE: Locus of enterocyte effacement

Qty: Quantity

***Stx* :**Shigatoxin gene

TSBYE: Tryptone Soya Broth-Yeast Extarct

XLD: Xylose-Lysine Deoxycholate

MSA: Mannitol Salt Agar

***mdh* :** Malate dehydrognase gene

LT: heat-labile enterotoxin

ST : Heat-stable enterotoxin

VT: Verocytotoxin

Eae: Attaching and effacing gene

BFP: Bundle-forming pilus

CFU: Colony forming unit

FemA: factor essential for expression of methicillin resistance

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Abstract

Foodborne diseases occur worldwide, including those acquired through the consumption of contaminated meat. This study was conducted to investigate the prevalence of enterotoxigenic *Staphylococcus aureus*, *Salmonella* and *Escherichia coli* pathotypes contamination in 40 samples of fresh (n=35) and frozen (n=5) beef, turkey and chicken using multiplex PCR. The meat samples were purchased from local markets in Jenin district, Palestine. Results of this research showed that the total mesophilic aerobic bacterial counts ranged between 4.3 log₁₀ to 5.7 log₁₀ cfu/g for frozen meat and 6.95 log₁₀ to 7.78 log₁₀ cfu/g for fresh meat. Results showed that the prevalence of *S. aureus*, *Salmonella* and *E. coli* was 30%, 25% and 95%, respectively. It was found that 75% of *S. aureus* strains were enterotoxigenic. Two samples of non *S. aureus* (*FemA*⁻) were toxigenic one of them was *sec*⁺ and the other was *see*⁺. These results showed that 89.5% (34/38) of meat samples contaminated with *E. coli* were belonged to enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), diffuse adherent *E. coli* (DAEC) pathotypes. A total of 21%

(8/38) of meat samples contaminated with *E. coli* were coinfecting with these pathotypes.

It is recommended to establish a permanent program for surveillance of microbial contamination with all foodborne pathogens. This will generate and provide data with statistical and epidemiological value, and can be used for estimating the exposure of consumers to foodborne pathogens and evaluating the effects of control measures on the contamination of food.

Chapter One

Introduction

1.1 General background

Food is considered the most important energy source for humans and animals, may be easily contaminated with pathogens if not handled appropriately (Mead *et al.*, 1999). The World Health Organization (WHO) defines foodborne illnesses as diseases, usually either toxic or infectious in nature, caused by agents that enter the human body through the process of food ingestion. The presence of living microorganisms in food is a natural and unavoidable occurrence. Cooking generally kills most of pathogenic microorganisms, but undercooked foods, processed ready-to-eat foods, and minimally processed foods can contain harmful bacteria that are serious health threats. More than 200 known diseases being transmitted through food, pathogens including bacteria, parasites, toxins, metals, and prions are the most significant cause. The symptoms of foodborne illness range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes (Mead *et al.*, 1999).

The spectrum of food-borne diseases is changing in constant rate. For example, a century ago, typhoid fever, tuberculosis and cholera were common food-borne diseases. Improvements in food safety, that included pasteurization of milk, safe canning, and disinfection of water supplies have eliminated those diseases. Today, other food-borne infections have taken their place, including some that have only recently been discovered.

Moreover, many emerging strains have been found to possess a combination of genetic traits that enhance virulence to cause disease in humans. This stands to that other pathogenic bacteria will become the subject of legislation in the future. The major problem in some pathogens such as *E. coli* O157:H7, is their ability to survive for long period under adverse conditions (Wilkes *et al.*, 2005; Uhlich *et al.*,2010, Van *et al.*, 2011). In addition, some salmonella serotypes have been reported to resist some therapeutic antibiotics (Antunes *et al.*, 2003).

The foundational knowledge of stress adaptation and long-term survival of some bacterial pathogens must be taken in to consideration. Investigation of the survival of foodborne pathogens would be beneficial to our understanding of transmission of these pathogens and the potential sources of foodborne illness (Allen *et al.*, 2008; Shen *et al.*, 2011).

Food safety is a global health goal and the food-borne diseases take a major crisis on health. The detection and enumeration of microbial pathogens in food and on surfaces that come into contact with food is the major step in the prevention and recognition of problems related to health and any integrated food safety program. Both food industrial companies and government authorities use chemical and microbiological analysis to monitor and control the state of contamination and the quality at all times and analyze trends so as to assess and detect emerging risks (López-Campos *et al.*, 2013).

Recently, an increasing number of countries in the Eastern Mediterranean region have moved to improve, update and strengthen their systems and infrastructure for food safety. These countries have adopted an approach based on risk management to monitor and control the safety of domestically produced and imported food or drafted new food legislation. Some countries have well-functioning foodborne surveillance systems and reporting mechanisms. Given the strong reliance of the Eastern Mediterranean region on food imports, ensuring the safety and quality of imported food is a recognized concern throughout the region. Many countries in the region have embarked on unifying food safety activities from farm to fork. They have established or are establishing food and drug authorities, which will cover food laws and regulations, food control management, foodborne disease surveillance and investigation systems, inspection services, recall and tracking systems, food monitoring laboratories, and information and education activities for the consumers themselves (Elmi, 2004).

Microbiological analysis of foods is based on the detection of microorganisms by conventional and standard bacterial detection methods such as culture and colony counting methods, immunology-based methods and DNA-based methods, may need up to several hours or even a few days to obtain the results (Velusamy et al., 2010; López-Campos *et al.*, 2012). Due to the perishable nature of most food products, there is an increased demand for the development of rapid, specific, sensitive, accurate and field-applicable methods to detect microorganisms. In particular, tests that

can be sensitive and completed within short time that would enable processors to take quick corrective actions when contaminants are detected. The use of DNA based methods in microbial diagnostic has greatly enhanced the ability to investigate and quantify particularly pathogenic bacteria in both food and water. Many of these molecular techniques have been accepted and implemented in standard protocols for detection and quantification of the most important pathogens (Beneduce *et al.*, 2007).

Chapter Two

Literature review

2.1. Overview of food-borne illness:

The WHO reported in 2005 alone, that 1.8 million people died from diarrhoeal diseases and a high proportion of these cases can be referred to contamination of food and drinking water (WHO, 2008). Of the known pathogens that cause foodborne illness *Aeromonas hydrophila*, *Bacillus cereus*, *Brucella* spp, *Campylobacter* spp, *Clostridium* spp, *Escherichia coli*, *Listeria monocytogenes*, *Mycobacterium bovis*, *Salmonella* spp, *Shigella* spp, *Staphylococcus aureus*, *Vibrio* spp and *Yersinia enterocolitica*. There are possibilities of other newly emerging foodborne diseases (WHO, 2008). A number of factors can explain the emergence of new foodborne pathogens such as new animal feeding practices, changes in animal husbandry, increase in international trade, changes in the agronomic process, changes in food technology, increase in travel, changes in lifestyle and consumer demands and increase in susceptible populations (Elmi, 2004). In developed countries it is estimated that up to a third of the population are affected by microbiological foodborne illnesses each year (De Guisti *et al.*, 2007). In the United States, foodborne diseases caused by 31 known pathogens are responsible for an estimated 9.4 million episodes of foodborne illness, that 5.5 million (59%) foodborne illnesses were caused by viruses, 3.6 million (39%) by bacteria, and 0.2 million (2%) by parasites. Foodborne infections caused 56 000 hospitalizations, and 1300 deaths each year (Scallan *et al.*, 2011). The CDC estimates that 47.8

million Americans (roughly 1 in 6 people) are sickened by foodborne disease every year (CDC, 2011). It is estimated that 130 million Europeans (WHO 2000), and 5.4 million Australians are annually affected by episodes of foodborne disease (Hall et al., 2005).

Approximately, 98% of microbes found in food commodities are non-pathogenic (Kumar *et al.*, 2002). Some pathogenic microorganisms responsible for foodborne illness are listed in Table 1. *Vibrio* spp. and *Aeromonas* spp. are normal inhabitants of aquatic environments and some species are recognized as human pathogens causing enteric pathologies, wound infection and septicemia (Thompson *et al.*, 2004; Janda and Abbott, 2010). *Vibrio cholerae* and non-epidemic *Vibrio* spp., including *V. parahaemolyticus* and *V. vulnificus*, are associated with the consumption of raw or undercooked shellfish or exposure of skin wounds to water (Morris, 2003).

Among various food-borne pathogens, *Bacillus cereus* and *Staphylococcus aureus* has been extensively reported in different types of food (Duc *et al.*, 2005; Pinto *et al.*, 2005; Shaheen *et al.*, 2006; King *et al.*, 2007; Svensson *et al.*, 2007; Awny *et al.*, 2010). *Bacillus cereus* causes two distinct food poisoning syndromes, diarrheal and emetic food poisoning, as well as a variety of typically necrotic non gastro-intestinal infections (Callegan *et al.*, 2002; Schoeni and Wong, 2005). Listeriosis is an emerging zoonotic infection of humans and ruminants worldwide caused by *Listeria monocytogenes*. Epidemiological studies have indicated that

both epidemic and sporadic cases of human listeriosis occur following consumption of contaminated food. Since 1980s, the incidence has risen steadily including large outbreaks making listeriosis one of a major public health issue, leading in intensified surveillance and control of *Listeria monocytogenes* in food industry, which contributed to a decrease of human listeriosis cases. Unlike infection with other common foodborne pathogens, listeriosis is associated with the highest case fatality rate ranges from 24% to 52% despite adequate antimicrobial treatment (Oevermann *et al.*, 2010).

Some foodborne diseases are well recognized, but are considered emerging because they have recently become more common. Though there are various foodborne pathogens that have been identified for foodborne illness, *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 have been generally found to be responsible for majority of food-borne outbreaks (Alocilja and Radke, 2003; Chemburu *et al.*, 2005). Data on foodborn illnesses in USA showed that, most (58%) illnesses were caused by norovirus, followed by nontyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (9%). Leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *Toxoplasma gondii* (8%). Leading causes of death were nontyphoidal *Salmonella* spp. (28%), *T. gondii* (24%), *L. monocytogenes* (19%), and norovirus (11%) (Scallan *et al.*, 2011).

In Palestine, a total of 250 stool samples were collected during an outbreak from symptomatic and asymptomatic patients in northern Palestine in 1999. A total of 176 (70.4%) were identified as Shiga toxin-producing *Escherichia coli* (STEC), of the 176 STEC isolates, 124 (70.5%) were of serotype O157 (Adwan et al., 2002). Also, 14.4% raw beef samples in northern Palestine during 2001 were contaminated with STEC (Adwan and Adwan, 2004). In addition, 150 children less than 5 years of age suffering from acute gastroenteritis and diarrhea were investigated for various enteropathogens by conventional and molecular techniques. Bacterial enteropathogens were detected in 17.3% of the diarrheal samples. *Shigella* spp was the most common bacterial pathogen (6.0%), followed by *Campylobacter coli/jejuni* (4.7%), *Escherichia coli* O157:H7 (4.7%), and *Salmonella* spp (2.0%) (Abu Elamreen et al., 2007).

Various meat or animal products such as beef, eggs, dairy, fish, and poultry are important reservoirs for many of the food-borne pathogens and have been associated with largest number of foodborne diseases outbreaks during 2009–2010. *Salmonella* in sprouts and vine-stalk vegetables are responsible for foodborne diseases outbreaks. The large number of outbreaks caused by unpasteurized dairy products is consistent with findings that more outbreaks occur in states that permit the sale of unpasteurized dairy products (MMWR, 2013).

2.1.1 *Salmonella*

Salmonella serotypes are ubiquitous enteric bacteria and etiological agents of food-borne gastroenteritis (salmonellosis), causing typhoid and paratyphoid fevers. There are more than 2500 serovars of *Salmonella* and all are potential pathogens (Chattopadhyay *et al.*, 2013). Biologically, *Salmonella* is very similar to *E. coli* in that it is a Gram-negative facultative anaerobe that colonizes the intestinal tracts mainly human or animal host. The bacteria are transmitted to humans through consumption of contaminated food of animal origin, mainly meat, poultry, eggs and milk (Jay *et al.*, 2005). The symptoms of *Salmonella* infection usually appear 12–72 hours after infection, and include fever, abdominal pain, diarrhea, nausea and sometimes vomiting (Scallan *et al.*, 2011).

2.1.2 *Escherichia coli*

capable of causing intestinal disease. These bacteria include strains of enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), diffuse adherent *E. coli* (DAEC), and enteroinvasive *E. coli* (EIEC) (Cocolin *et al.*, 2000). Transmission of *E. coli* pathotypes appears to be mainly caused by consumption of foods of animal source, especially ground beef and milk. In addition, other foods implicated in outbreaks include turkey sandwiches, potatoes, roast beef, dry sausage, yogurt and apple cider (Deng *et al.*, 1996). Selected characteristics of each one of the groups is presented in Table 2.

Escherichia coli O157:H7 is a gram-negative, flagellated, rod-shaped bacteria. The cell wall contains the “O” antigen, and the “H” represents flagellar antigen. *E. coli* O157:H7 is specifically adapted for survival in the gastrointestinal tract of host organisms (Jay et al., 2005). It is most common as a causative agent of Hemolytic Uremic Syndrome (HUS). The virulence factor is due to production of shiga-like toxins, responsible for HUS which attack renal cells, causing lysis, that leading to bloody stool. Also, this pathogen is able to survive in a wide range of conditions including lower temperatures associated with storage of meat products. Scallan et al. (2011) reported that *E. coli* O157:H7 could be responsible for over 60,000 cases of illness each year and being responsible for up to 20 deaths on average.

2.1.3 *Staphylococcus aureus*

S. aureus is recognized worldwide as a frequent cause of foodborne disease in dairy foods, which produces a spectrum of proteins and virulence factors that are thought to contribute to its pathogenicity. Poultry, meat and egg products as well as milk and milk products have been reported as common foods that may cause staphylococcal food poisoning. Staphylococcal enterotoxins (SEs) are recognized agents of staphylococcal food poisoning syndrome, with sequelae such as shock, and may be involved in other types of infections in humans and animals (Adwan *et al.*, 2005; 2008; 2013).

Table 1. Some pathogenic microorganisms responsible for foodborne illness (Velusamy et al., 2010).

Pathogen	Associated foods (reported food contaminants)	Infective dose^a (no. of organisms)	Incubation period^b	Symptoms	Name of the disease
<i>Campylobacter jejuni</i>	Raw milk, and raw or under-cooked meat, poultry or shellfish	400–500	2 to 5 days	Fever, headache, and muscle pain followed by diarrhea, abdominal pain and nausea	Campylobacteriosis
<i>Salmonella</i> spp.	Raw/undercooked eggs, poultry, and meat; raw milk and dairy products; seafood; chocolate; salad and spices	15–20	12 to 24 h	Stomach pain, diarrhea, nausea, chills, fever, and headache	Salmonellosis
<i>E. coli</i>	Raw/undercooked eggs, poultry, and meat; raw milk and dairy products; seafood; and leafy vegetables	<10	2 to 4 days	Stomach pain, diarrhea, nausea, chills, fever, and headache	Hemorrhagic colitis
<i>L. monocytogenes</i>	Soft cheese, raw milk, improperly processed ice cream, raw leafy vegetables; raw meat and poultry	<1000	2 days to 3 weeks	Fever, chills, headache, backache, sometimes abdominal pain and diarrhea	Listeriosis
<i>Bacillus cereus</i>	Meats, milk, vegetables, fish, rice, pasta, and cheese		30 min to 15 h	Diarrhea, abdominal cramps, nausea, and vomiting	Bacillus cereus food poisoning
<i>Clostridium botulinum</i>	Improperly canned foods, garlic in oil, and vacuum packaged and tightly wrapped food	< nano grams	12–36 h	Double vision, droopy eyelids, trouble speaking and swallowing, and difficulty breathing	Foodborne botulism
<i>Clostridium perfringens</i>	Undercooked meats, meat products, and gravies	>10 ⁸	8–22 h	Abdominal cramps and diarrhea	Perfringens food poisoning
<i>Shigella</i>	Salads, raw vegetables,	<10	12–50 h	Abdominal pain, cramps,	Shigellosis

	dairy products, and poultry			fever, vomiting, and diarrhea containing blood and mucus	
<i>Yersinia enterocolitica</i>	Meat (mostly pork), oysters, fish, and raw milk	Unknown	1–3 days	Diarrhea and/or vomiting; fever and abdominal pain	Yersiniosis
<i>Vibrio parahaemolyticus</i>	Raw, improperly cooked, or cooked, recontaminated fish and shellfish, and oysters	> 1 million	4 h–4 days	Diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills	V. parahaemolyticus associated gastroenteritis
<i>Vibrio vulnificus</i>	Raw or recontaminated oysters, clams, and crabs	<100	<16 h	Diarrhea, and wound infections	Syndrome called “primary septicemia

^a **Infective dose:** the amount of agent that must be consumed to give rise to symptoms of foodborne illness.

^b **Incubation period:** the delay between consumption of a contaminated food and appearance of the first symptoms of illness.

Table 2. Selected characteristics of pathogenic *E. coli* groups.

	Foodborne	Disease	Invasion	Enterotoxin	Infectious dose
EPEC(1)	Any food exposed to fecal contamination	Infantile diarrhea	Locus of enterocyte effacement (LEE), induce AE lesions	Unrelated to the excretion of typical <i>E. coli</i> enterotoxins	Low in infants, but >10 ⁶ cells in adults
EHEC(1)	Various food implicated Hemorrhagic colitis	complications can lead to Hemolytic Uremic Syndrome	Locus of enterocyte effacement (LEE) induce AE lesions	Large qty of Shigatoxin (Stx)/ verotoxin	Suspected as few as 10 cells
STEC(3)	Contamination can occur during the processing of slaughtered animals at abattoirs especially in the case of ground beef.	Hemolytic Uremic Syndrome	Absence of locus of enterocyte effacement (LEE)	Large qty of Shigatoxin (Stx)/ verotoxin	
EIEC(1)	Any food exposed to human feces from an ill individual	Bacillary dysentery	Invades and proliferates in colonic epithelial cells similar to shigellae	Unrelated to the excretion of typical <i>E. coli</i> enterotoxins	10 cells
ETEC(1)	Unclear	Gastroenteritis in infants and adult travelers	Fimbrial attachment and colonization of colonic cells	Yes (heat labile LT and heat stable ST)	10 ⁶ -10 ⁹ cells
EAHEC (1,2)	Associated with undercooked beef	Hemolytic Uremic Syndrome	Absence of locus of enterocyte effacement (LEE)	Large qty of Shigatoxin (Stx)/ verotoxin	
DAEC(1) factor	Unclear	Infantile diarrhea	No invasion to epithelial cells, no adherence factor		
EAEC(2)	Unclear	Persistent diarrhea infants and children	Aggregative adherence to Hep-2 cells in stacked brick fashion		

(1) (Kaper et al., 2004) (2) (Elzbieta Brzuszkiewicz et al., 2011). (3) (Angela M et al., 2011)

2.2. Molecular analysis methods:

Detection, isolation and identification of different types of microbial pathogens contaminating food would be time consuming and expensive if done in a conventional way. Usually, detection of pathogenic bacteria using conventional methods is largely based on cultivation procedures, which use enrichment broths followed by isolation of colonies on selective media, biochemical identification and confirmation of pathogenicity of the isolates. However, these methods have several limitations, such as dependency on enrichment and selective culture, difficulty of quantitative analysis and long culture time. The development of rapid and reliable detection methods for food-borne pathogens is ongoing to complement or replace culture-based approaches and bypass some of their intrinsic biases and their own limitations. Examples of these methods include biosensors (e.g., bioluminescence biosensor, impedimetry, piezoelectric biosensors, etc), immunological methods, and nucleic acid based assays (Mandal *et al.*, 2010). PCR offers distinct advantages over culture and other standard methods such as specificity, sensitivity, rapidity, accuracy and capacity to detect small amounts of target nucleic acid in a sample. PCR based methods are used in the detection of wide range of pathogens like *S. aureus*, *L. monocytogenes*, *Salmonella*, *B. cereus*, *E. coli* O157: H7, *Yersinia enterocolitica*, *C. jejuni* (Velusamy *et al.*, 2010). Multiplex PCR is very useful technique as it allows the simultaneous detection of several pathogens by introducing different primers to amplify DNA regions coding for specific genes of each bacterial strain targeted (Touron *et al.*, 2005). A

multiplex PCR method was developed to rapidly detect different food bacterial pathogens (Wang *et al.*, 1997; Cocolin *et al.*, 2000; Beneduce *et al.*, 2007; kim *et al.*, 2007; Jeshveen *et al.*, 2012; Kawasaki *et al.*, 2009). Examples of multiplex PCR technique for the simultaneous detection pathogens include multiplex PCR assay for rapid and simultaneous detection of *E. coli* O157:H7, *Salmonella* and *Shigella* (Li *et al.*, 2005), simultaneous detection of *E. coli* O157:H7, *Salmonella*, *S. aureus*, *L. monocytogenes*, and *V. parahaemolyticus* (Kim *et al.*, 2007), simultaneous detection of bacteria of the genus *Listeria*, *L. monocytogenes*, and major serotypes and epidemic clones of *L. monocytogenes* (Chen and Knabel, 2007), simultaneous detection of *E. coli* O157: H7 and *L. monocytogenes* (Mukhopadhyay and Mukhopadhyay, 2007), simultaneous detection of *Listeria*, *Salmonella* and *E. coli* pathogens (Tavakoli *et al.*, 2010), simultaneous detection of *Salmonella* spp., *L. monocytogenes*, *Escherichia coli* O157:H7, and *S. aureus* (Kawasaki *et al.*, 2009; 2012). In spite of its advantages, from an industrial point of view routine detection of microbes using PCR can be expensive and complicated, requiring skilled workers to carry out the tests.

2.3 Aims of the study:

Food safety is a global health goal and the foodborne diseases take a major crisis on health. *Salmonella* spp, *E. coli*, and *S. aureus* are major foodborne pathogens that represent a permanent challenge to the meat industry. These bacterial species are considered dangerous pathogens with their ability to cause diseases in humans and animals. Detection and

identification of microbial pathogens in food is the solution to the prevention and recognition of problems related to health and safety. This study aimed to detect enterotoxigenic *S. aureus*, *Salmonella* spp and *E. coli* pathotypes from meat products (fresh and frozen) using PCR technique and to enumerate bacterial cells in these food samples. The meat samples included in this study were collected from local markets in Jenin district, Palestine.

Chapter Three

Materials and Methods

3.1. Collection of samples

Forty meat samples, included fresh (35 samples: 13 beef; 13 chicken and 9 turkey) and frozen (5 samples: 2 beef ; 2 chicken and 1 turkey) were purchased randomly during May-June 2014, from different localities in Jenin governorate as shown in (Figure 1). These samples were transferred in container, under aseptic conditions provided with ice bags within few hours to the Microbiology Laboratory, Department of Biology at An-Najah National University-Nablus, Palestine.

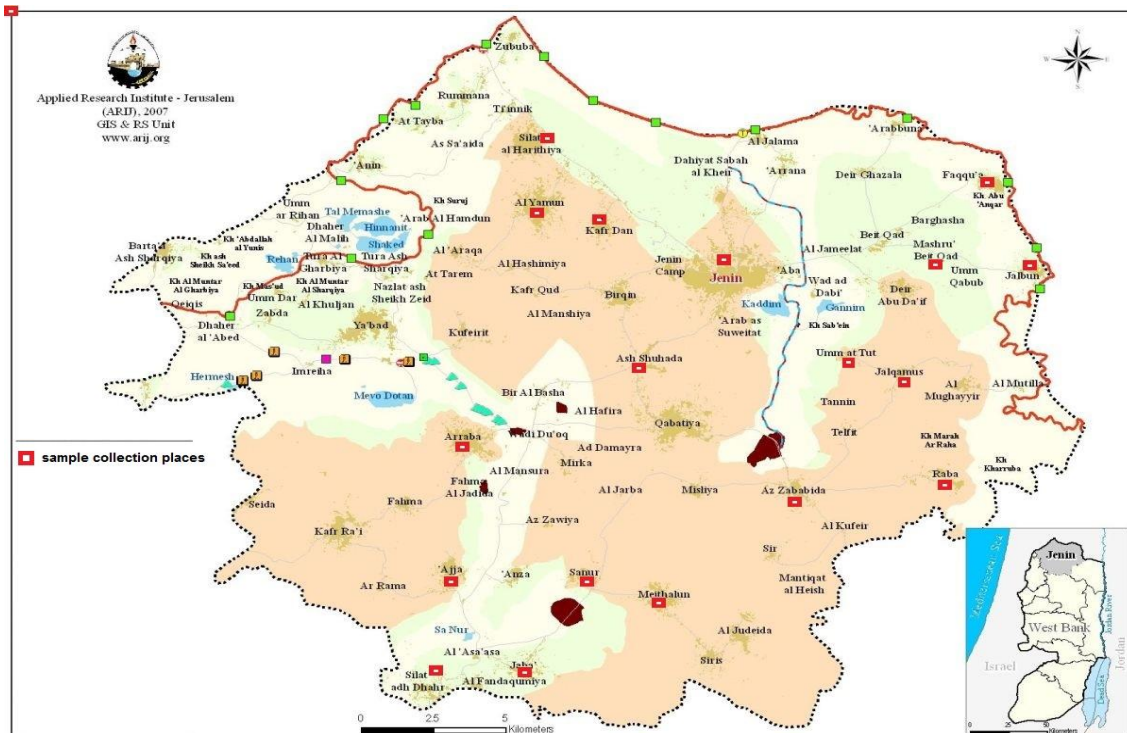


Figure 1. Distribution of samples collection localities in Jenin governorate.

3.2. Media preparation

3.2.1 Tryptone Soya Broth-Yeast Extarct (TSBYE) media:

TSBYE was prepared according to the following formula; Tryptone Soya Broth (Oxoid) (30 g) and Yeast Extarct (Acumedia) (6 g) were suspended together in one liter of distilled water, mixed well. The broth was then distributed into flasks to have 90 ml each. The flasks were autoclaved at 121°C for 15 minutes, allowed to cool and then stored at 4°C.

3.2.2 Xylose-Lysine Deoxycholate (XLD) Agar:

XLD Agar (Oxoid, UK) was prepared according to manufacturer's instructions labeled on the bottle. In a 2 L flask, 1 L of deionized water was mixed with 56.7 g XLD Agar, heated and stirred until the agar dissolved. The solution allowed to boil for 1 minute, and then autoclaved at 121°C for 15 minutes. After that it was allowed to cool to about 50°C, the agar was poured into sterile Petri dishes to have 25-30 ml each that was left overnight at room temperature. The following morning the Petri dishes were turned upside down and stored at 4°C.

3.2.3 MacConkey Agar:

MacConkey agar (HIMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A 1L flask containing 500 ml deionized water and 25 g MacConkey agar was heated and stirred until the agar dissolved. The solution was allowed to boil for 1min, and then was autoclaved at 121°C for 15 min. After that it was allowed to cool,

and the agar was poured into sterile Petri dishes to have 20 ml that was covered and left overnight. The following morning the Petri dishes were turned upside down and stored at 4°C.

3.2.4 Mannitol Salt Agar (MSA):

BBL™ Mannitol agar (BD) was prepared according to the manufacturer's instructions labeled on the bottle. In a 1 L flask, 500 ml deionized water were heated and mixed with 55.5 g MSA until the agar dissolved. The solution was allowed to boil for 1 minute, and then autoclaved at 121°C for 15 minutes. After that it was allowed to cool to about 50°C, and poured into sterile Petri dishes to have 20 ml each, then left overnight at room temperature. The following morning the Petri dishes were turned upside down and stored at 4°C.

3.3. Food sample preparation bacterial culturing

Of each food sample purchased from local food stores, 10 g were homogenized in 90 ml TSBYE medium, then the suspension was mixed well. Five of serial decimal dilutions of samples with sterile normal saline were in duplicates on nutrient agar. The plates were then incubated at 37°C for 24h before colonies were counted. Then 5 ml of TSBYE was incubated at 37°C/18-24h and used for DNA extraction and subcultured on XLDA, MSA and MacConkey.

3.4. DNA extraction:

DNA was prepared for PCR according to the method described previously with some modifications (Adwan et al., 2013). Briefly, 1.5 ml of cells from overnight TSBYE broth were centrifuged, the pellet was washed twice with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), then resuspended in 0.5 ml of sterile distilled H₂O, and was boiled for 10-15 min. The cells then immediately were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X *g* for 5 min. DNA was extracted from the supernatant using phenol-chloroform method, then DNA was precipitated using 96% cold ethanol. The nucleic acid pellet was washed with 70% cold ethanol, dried and then resuspended in 300 µl TE (Tris 10 mM, EDTA 1 mM, pH 8), DNA concentration was determined using a spectrophotometer and the samples were stored at -20°C until use for further DNA analysis.

3.5. Detection of food pathogens by PCR

3.5.1 Detection of *E. coli* *Mdh* gene:

E. coli were identified by PCR with specific primers for malate dehydrogenase gene (*mdh*) as described previously (Hsu *et al.* 2007). Primer nucleotide sequences and expected size of amplicon are presented in Table 3. The PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix with MgCl₂ (ReadyMix™ Taq PCR Reaction Mix with MgCl₂, Sigma), 0.4 µM of each primer, and 2 µL DNA template. DNA amplification was performed using thermal cycler (Mastercycler Personal, Eppendorf)

according to the following thermal conditions: initial denaturation for 2 min at 94°C followed by 30 cycles at 94°C for 1 min for denaturation, annealing at 59°C for 30 s and extension at 72°C for 1 min. Final extension was carried out at 72°C for 5 min. The amplified products were examined by (2%) agarose gel electrophoresis to determine the size of amplified fragment for each isolate. A DNA ladder of 100 bp was also included in all gels (100bp DNA ladder RTU, GeneDireX). Negative control was included in these experiments.

3.5.2 Detection of *femA* (*S. aureus*) and 1.8-kb *HindIII* DNA fragment (*Salmonella* spp.):

The primers targeted a *Salmonella* species-specific sequence within a 1.8-kb *HindIII* DNA fragment, and the *S. aureus femA* gene and expected sizes of amplicons are presented in Table 3. PCR was performed as described previously with some modification (Kawasaki et al., 2012). The PCR reaction mix (25 µL) was performed using 12.5 µL of PCR premix with MgCl₂ (ReadyMix™ Taq PCR Reaction Mix with MgCl₂, Sigma), 0.4 µM of each primer, and 2 µL DNA template. DNA amplification was performed using thermal cycler (Mastercycler Personal, Eppendorf) according to the following thermal conditions: initial denaturation for 2 min at 94°C followed by 40 cycles at 94°C for 20 s for denaturation, annealing at 56°C for 30 s and extension at 72°C for 30 s. Final extension will be carried out at 72°C for 5 min. The amplified products were examined by 1.5% agarose gel electrophoresis to determine the size of amplified fragment for each isolate. A DNA ladder of 100bp was also

included in all gels (100bp DNA ladder RTU, GeneDireX). Negative control was included in these experiments.

3.5.3. Detection of staphylococcal enterotoxin (*sea-see*) genes:

Primer nucleotide sequences and expected sizes of amplicons are presented in Table 3. The PCR reaction mix (25 μ L) was performed using 12.5 μ L of PCR premix with MgCl₂ (ReadyMix™ Taq PCR Reaction Mix with MgCl₂, Sigma), 0.4 μ M of each primer, and 2 μ L DNA template.

DNA amplification was performed using thermal cycler (Mastercycler Personal, Eppendorf) as the following thermal conditions: initial denaturation for 2 min at 94°C followed by 30 cycles at 94°C for 1 min for denaturation, annealing at 55°C for 1 min and extension at 72°C for 2 min. Final extension was carried out at 72°C for 5 min. The amplified products were examined by 1.5% agarose gel electrophoresis to determine the size of amplified fragment for each isolate. A DNA ladder of 100bp was also included in all gels (100bp DNA ladder RTU, GeneDireX). Negative control was included in these experiments.

3.5.4. Detection of *E. coli* pathotypes:

The targeted genes from different *E. coli* pathotypes including enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC) and diffusely adherent *E. coli* (DAEC) were amplified using oligonucleotide primer pairs and expected sizes of amplicons are listed in Table 3. These

genes were detected with some modifications as described previously (Gómez-Duarte et al., 2009). The PCR reaction mix (25 μ L) was performed using 12.5 μ L of PCR premix with $MgCl_2$ (ReadyMixTM Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.4 μ M of each primer and 2 μ L DNA template. DNA amplification was performed using thermal cycler (Mastercycler Personal, Eppendorf) according to the following thermal conditions: initial denaturation for 2 min at 94°C followed by 40 cycles at 92°C for 30 s for denaturation, annealing at 59°C for 30 s and extension at 72°C for 30 s. Final extension was carried out at 72°C for 5 min. The amplified products were examined by (2%) agarose gel electrophoresis to determine the size of amplified fragment for each isolate. A DNA ladder of 100bp was also included in all gels (100bp DNA ladder RTU, GeneDireX).

Table 3. Target genes for PCR amplification, amplicon size, primer sequences and annealing temperature.

Organism	Gene target	Oligonucleotide sequence (5→3)*	Amplicon Size (bp)	Annealing temperature	Reference	Primer mix
<i>E. coli</i>	<i>Mdh</i>	Mdh1 ACT GAA AGG CAA ACA GCC AGG C mdh2 CGT TCT GTT CAA ATG CGC TCA GG	392	59°C	Hsu <i>et al.</i> 2007	1
<i>Salmonella</i> spp.	<i>HindIII</i> DNA fragment	TS-11 GTCACGGAAGAAGAGAAATCCGTACG TS-5 GGGAGTCCAGGTTGACGGAAAATTT	375	56°C	Tsen <i>et al.</i> 1994	2
<i>S. aureus</i>	<i>FemA</i>	Fem F TATGAGTTAAAGCTTGCTGAAGGTT Fem R TTACCAGCATTACCTGTAATCTCG	296	56°C	Kawasaki <i>et al.</i> , 2012	2
ETSA	<i>sea</i>	SEA-3 CCTTTGGAAACGGTTAAAACG SEA-4 TCTGAACCTTCCCATCAAAAAC	127	55°C	Becker <i>et al.</i> 1998	3
ETSA	<i>seb</i>	SEB-1 TCGCATCAAACCTGACAAACG SEB-4 GCAGGTACTCTATAAGTGCCTGC	477	55°C	Becker <i>et al.</i> 1998	3
ETSA	<i>sec</i>	SEC-3 CTCAAGAAGTACATATAAAAGCTAGG SEC-4 TCAAAATCGGATTAACATTATCC	271	55°C	Becker <i>et al.</i> 1998	3
ETSA	<i>sed</i>	SED-3 CTAGTTTGGTAATATCTCCTTTAAACG SED-4 TTAATGCTATATCTTATAGGGTAAACATC	319	55°C	Becker <i>et al.</i> 1998	3
ETSA	<i>see</i>	SEE-3 CAGTACCTATAGATAAAGTTAAAACAAGC SEE-2 TAACTTACCGTGGACCCTTC	178	55°C	Becker <i>et al.</i> 1998	3
EHEC	VT	VTcom-u GAGCGAAATAATTTATATGTG VTcom-d TGATGATGGCAATTCAGTAT	518	59°C	Gómez-Duarte <i>et al.</i> , 2009	4
EHEC, EPEC	<i>eae</i>	eae1 CTGAACGGCGATTACGCGAA eae2 CGAGACGATACGATCCAG	917	59°C	Gómez-Duarte <i>et al.</i> , 2009	4
EPEC	<i>bfpA</i>	BFP1 AATGGTGCTTGCGCTTGCTGC BFP2 GCCGCTTTATCCAACCTGGTA	326	59°C	Gómez-Duarte <i>et al.</i> , 2009	4
EAEC	<i>aggR</i>	aggRks1 GTATACACAAAAGAAGGAAGC aggRks2 ACAGAATCGTCAGCATCAGC	254	59°C	Gómez-Duarte <i>et al.</i> , 2009	4
ETEC	<i>LT</i>	LT1 GCACACGGAGCTCCTCAGTC LT2 TCCTTCATCCTTTCAATGGCTTT	218	59°C	Gómez-Duarte <i>et al.</i> , 2009	5
ETEC	<i>ST</i>	ST1 GCTAAACCAGTAGAG(C)TCTTCAAAA ST2 CCCGGTACAG(A)GCAGGATTACAACA	147	59°C	Gómez-Duarte <i>et al.</i> , 2009	5
DAEC	<i>daaE</i>	daaE1 GAACGTTGGTTAATGTGGGGTAA daaE2 TATTCACCGGTCGGTTATCAGT	542	59°C	Gómez-Duarte <i>et al.</i> , 2009	5

* All the primers used in this study were synthesized by Sigma-Aldrich (Israel).

Chapter Four

Results

4.1. Bacterial enumeration and cultural characterization

The total aerobic bacterial counts ranged between $4.3 \log_{10}$ to $5.7 \log_{10}$ cfu/g for frozen meat and $6.95 \log_{10}$ to $7.78 \log_{10}$ cfu/g for fresh meat. Results of bacterial culture on MacConkey agar showed that 95% (38/40) of samples were lactose fermenter with bright pink color colonies and not mucoid. On XLD Agar, 22.5% (9/40) of samples had colonies with black centers due to H_2S production. Results also showed that 65% (26/40) of samples subcultured on MSA were mannitol fermenter and had a yellow color due to acid production. Results of cultural characterization on different selective media; MacConkey agar, XLD Agar and MSA are represented in diagram 1.

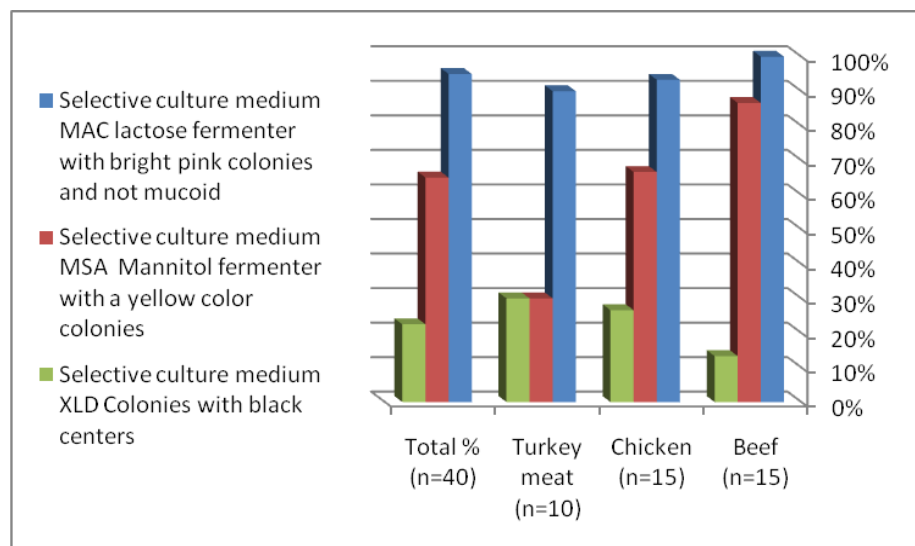


Diagram 1. Morphological characterization on different selective and differential media; MacConkey agar, XLD Agar and MSA.

4.2. Detection of *E. coli* by PCR

All of the 40 DNA samples were tested using primers specific for malic acid dehydrogenase (*mdh*) gene for *E. coli*, 95% (38/40) generated the expected PCR products with size equals to 392 bp. The prevalence of *E. coli* was 100%, 93.3% and 90% in beef, chicken and turkey meat, respectively. Results about prevalence of *E. coli* using PCR in three types of meat are presented in diagram 2 and Figure 2.

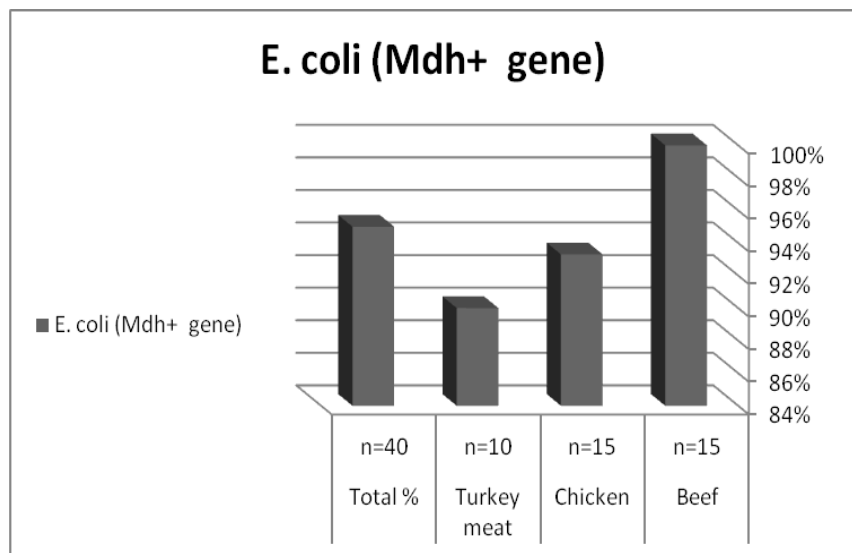


Diagram 2. Prevalence of *E. coli* in beef, chicken and turkey meat.

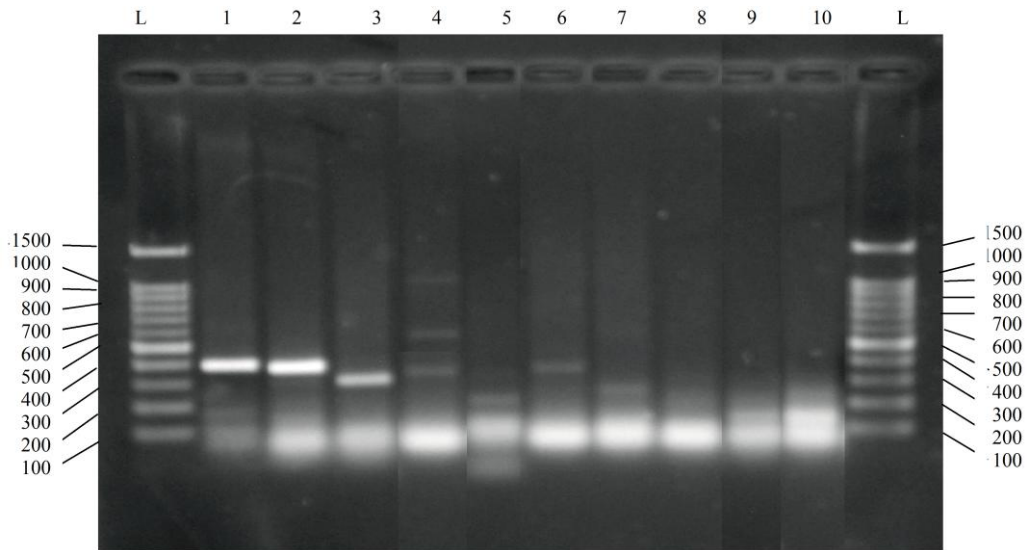


Figure 1: Multiplex PCR profiles. Lane L contained ladder (100bp DNA ladder RTU, GeneDireX), lane 1 represents *E. coli* target gene (*mdh*); lane 2 represent *Salmonella* spp. target gene (*HindIII* DNA fragment); lane 3 shows *S. aureus* target gene (*FemA*); lane 4 belonged to pathogenic *E. coli* groups target genes (*VT* for EHEC and *bfpA* for EPEC and *eae* for both); lanes 5 represents EAEC target gene (*aggR*); lanes 6-9 belonged to staphylococcal enterotoxin genes of (*sed*, *sec*, *see* and *sea*, respectively); lane 10 represents ETEC target genes (*LT* and *ST*).

4.3. Detection of *Salmonella* spp. and *S. aureus* by PCR.

PCR assay was carried out for the DNA from 40 meat samples to detect the presence of *Salmonella* spp. and *S. aureus* in three types of meat samples. The size of PCR products produced by specific primers for *Salmonella* spp. and *S. aureus* were 375 bp and 296 bp, respectively. The prevalence of *Salmonella* spp., *S. aureus* and both in forty meat samples was 17.5% and 22.5% and 7.5%, respectively. The distribution of *Salmonella* spp. and *S. aureus* in three types of meat products is presented in diagram 3 and Figure 1.

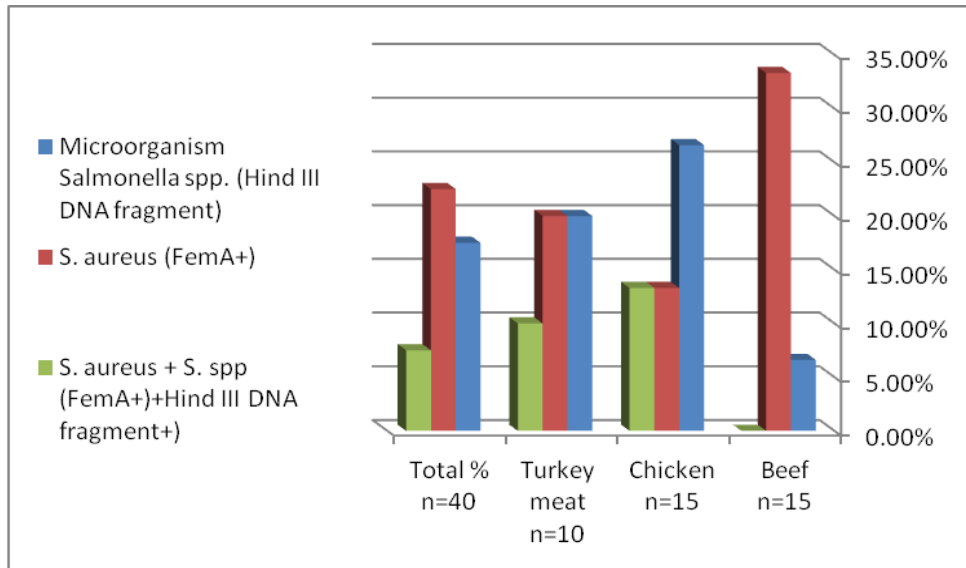


Diagram 3. Distribution of *Salmonella* spp and *S. aureus* in three types of meat products using PCR.

4.4. Detection of staphylococcal enterotoxin (*sea-see*) genes

PCR assay was conducted on DNA from all meat samples. Only staphylococcal enterotoxin genes were detected from samples contaminated with *S. aureus*. The prevalence of staphylococcal enterotoxin genes among *S. aureus* isolates was 25%, 0.0%, 0%, 8.3% and 25% for *sea*, *seb*, *sec*, *sed* and *see*, respectively. In addition, 5% of samples were coinfecting with *sea* and *see*. Two beef samples one was *sec*⁺ and the other *see*⁺, while these were *FemA*⁻ and non mannitol fermenter. Prevalence of staphylococcal enterotoxin genes among *S. aureus* isolates in meat samples are presented in diagram 4 and Figure 2.

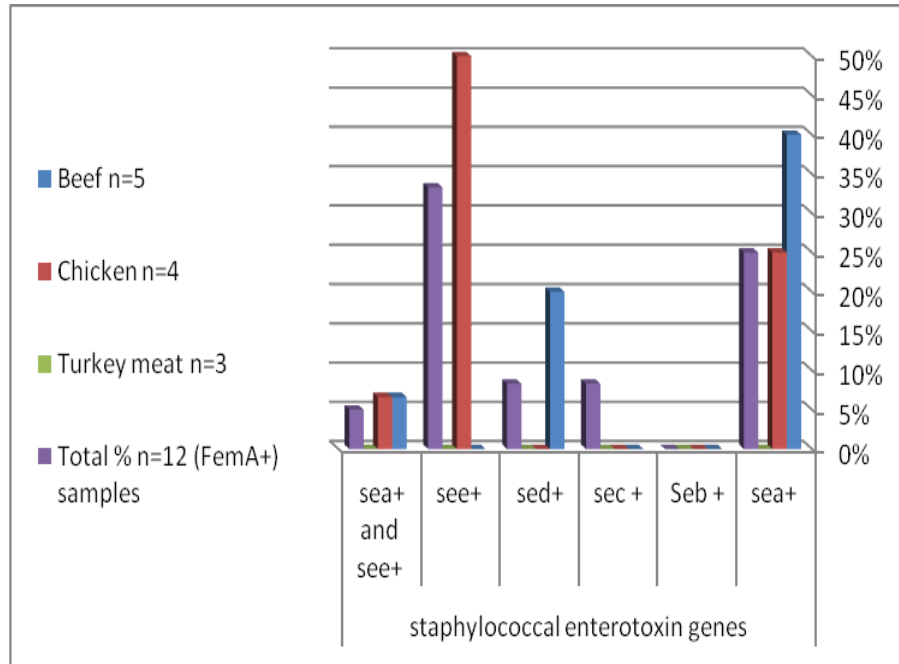


Diagram 4. Distribution of staphylococcal enterotoxin genes (*sea-see*) in meat samples.
 * Two samples one had *sec*⁺ and another had *see*⁺ but *FemA*⁻ and non mannitol fermenter.

4.5. Detection of *E. coli* pathotypes

The PCR assay was used for detecting the presence of pathogenic groups of *E. coli*. Detection of *VT* and *eae* genes for EHEC, *bfpA* and *eae* genes of EPEC, *aggR* gene for EAEC, *daaE* gene DAEC and *LT* and/or *ST* for ETEC. Amplification of these genes produced PCR products of 518 bp and 917 bp, 326 bp and 917 bp, 254 bp, 542 bp and 218 bp and/or 147 bp for EHEC, EPEC, EAEC, DAEC and ETEC, respectively. These results showed that 89.5% (34/38) of meat samples contaminated with *E. coli* belonged to *E. coli* pathotypes tested in this research. The total prevalence of uni-infected samples with EHEC, EPEC, EAEC, DAEC and ETEC was 0%, 0%, 5%, 0% and 60 % respectively, while 21% (8/38) of meat samples

contaminated with *E. coli* were coinfecting with these pathotypes.

Prevalence of *E. coli* pathotypes groups in 3 types of meat are presented in diagram 5, table 4 and figure 1.

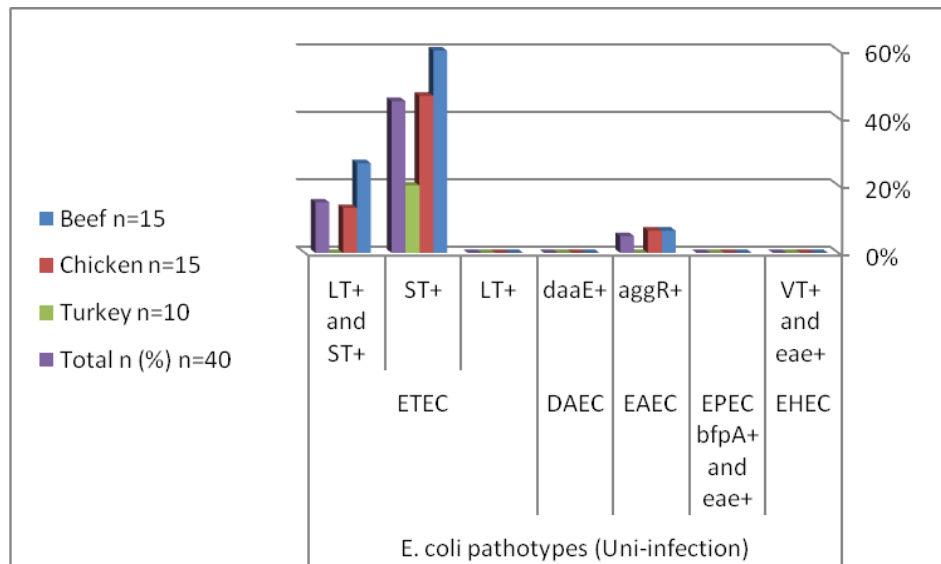


Diagram 5. Prevalence of *E. coli* pathotypes EHEC, EPEC, EAEC, DAEC and ETEC with uni-infection in 3 types of meat samples .

Table 4. Prevalence of *E. coli* pathotypes coinfection in 3 types of meat samples.

<i>E. coli</i> pathotypes					Source and number of samples			
EHEC (<i>VT⁺+eae⁺</i>)	EPEC (<i>bfpA⁺+eae⁺</i>)	ETEC		EAEC	Beef	Chicken	Turkey	Total
		<i>ST⁺</i>	<i>LT⁺</i>	<i>aggR⁺</i>				
+	-	+	-	-	1	1	0	2
-	+	+	+	-	1	0	0	1
-	-	+	+	+	0	1	0	1
+	-	+	-	+	1	0	0	1
+	-	-	-	+	1	0	0	1
+	+	-	-	-	2	0	0	2

+: presence of gene; -: absence of gene

Chapter Five

Discussion

Conventional detection of pathogenic bacteria is largely based on cultivation procedures, which use enrichment broths followed by isolation of colonies on selective media, biochemical identification and confirmation of pathogenicity of the isolates. Since many human pathogenic bacteria can be found in the contaminated food including meat, the specific detection of these pathogenic microorganisms in food is often suggested to assess a more accurate human health risk. Development of new techniques including molecular assays are used to complement or replace culture-based approaches and bypass some of their intrinsic biases and their own limitations. PCR is one of these techniques and is considered a sensitive detection method for specific pathogens and multiplex PCR assay may provide a useful tool for rapid and specific detection of pathogens in food. This is an important step towards the control and prevention of food-borne epidemics (Awny et al., 2010). Numerous studies were published about PCR detection of different food-borne pathogens (Li *et al.*, 2005; Beneduce *et al.*, 2007; kim *et al.*, 2007; Jeshveen *et al.*, 2012; Kawasaki *et al.*, 2009).

Results showed heavy bacteriological load in different meat types with a total viable counts ranging from 4.3 log₁₀ to 5.7 log₁₀ cfu/g for frozen meat and 6.95 log₁₀ to 7.78 log₁₀ cfu/g for fresh meat. The presence of a high number of viable bacteria is considered as an indicator of the short expected shelf life of meat. Total bacterial viable counts in different

types of meats ranges from 5.6 log₁₀ CFU/g to 9 log₁₀ CFU/g (Eisel et al., 1997; Bhandare et al., 2007; Arain et al., 2010; Awny et al., 2010; Abdellah et al., 2013; Anihouvi et al., 2013). The presence of high count of viable bacteria organisms from 6 log₁₀ CFU/g to 9 log₁₀ CFU/g, as an indication of open-air meat spoilage (Eribo and Jay, 1985). Therefore, it is considered that fresh meat that contains 5 log₁₀ CFU/g to 6 log₁₀ CFU/g of background organisms are inherently safer than those that contain less bioload; however, this hypothesis applies only to harmless bacteria (Jay, 1996).

A wide spectrum of pathogens play a role in foodborne disease. Most of them have a zoonotic origin and have reservoirs in healthy food animals from which they spread to an increasing variety of foods. Therefore, foods of animal origin are considered major vehicles for the transmission of human foodborne infections (Todd, 1997). *Salmonella* and *S. aureus* are the most common and frequent pathogens responsible for food poisoning and food related infections (Costa et al., 2012; Aydin et al., 2011). According to WHO (WHO, 2006), 25% of the diarrhea in foodborne illness is caused by food infected with *E. coli*.

S. aureus food poisoning is one of the most economically important food-borne pathogen worldwide. Results of this research showed that 30% of meat samples were contaminated with *S. aureus*, and 75% (9/12) were toxigenic. The prevalence of *S. aureus* in different food ranged from 12.12% to 50.8% (Adwan et al., 2005; Awny et al., 2010; Vázquez-

Sánchez et al., 2012; EI-Jakee et al., 2013). The prevalence of enterotoxigenic *S. aureus* in different food samples reported for countries such as Brazil, the USA, Switzerland, South Korea, Poland, Slovakia, China, France, Palestine, Egypt, Brasil, Thailand, Spain and Japan has ranged from 4.7% to 100% (Adwan *et al.*, 2005; Pelisser et al., 2009; Awny et al., 2010; Vázquez-Sánchez et al., 2012; EI-Jakee et al., 2013; Akbar and Anal, 2013; Anihouvi et al., 2013). Two samples which had *sec*⁺ or *see*⁺ but *FemA*⁻ and non mannitol fermenter, these were *Staphylococcus* coagulase-negative. It was reported that, coagulase-negative as well as coagulase-positive staphylococci are capable of genotypic and phenotypic enterotoxigenicity (Veras et al., 2008; Podkowik et al., 2013). In the present study, detection of toxin genes by PCR allows the determination of potentially enterotoxigenic pathogen irrespective of whether the strain produces the toxin or not. For this reason, PCR may be considered more sensitive than immunological methods that determine SE production (Adwan *et al.*, 2005). The most common types of staphylococcal enterotoxins are SEA to SEE. Isolates carrying toxin genes *sea* to *see* are responsible for 95% of staphylococcal food poisoning outbreaks (Bergdoll, 1983). SEA is the most common enterotoxin recovered from food poisoning outbreaks (Balaban and Rasooly, 2000). The remaining staphylococcal food-borne disease outbreaks may therefore be associated with other newly identified SEs (MacLauchlin et al., 2000; Rosec and Gigaud, 2002; Omoe et al., 2002). Staphylococcal enterotoxins are thermostable and also resistant to gastrointestinal proteases such as

pepsin, explaining its ability to remain active after ingestion. Therefore, the presence of *S. aureus* in food can be considered a potential health risk (Adwan et al., 2006).

Among various food-borne pathogens, *Salmonella* serotypes are the most common bacteria responsible for food-borne gastroenteritis. There are more than 2500 serovars of *Salmonella* and all are considered as pathogenic. Results of this research showed that 25% of meat samples were contaminated with *Salmonella*. The prevalence of *Salmonella* in different food ranged from 1.56% to 100% (Zhao et al. 2001; Rivera-Betancourt et al., 2004; Busani et al., 2005; Cohen et al. 2007; Bhandare et al., 2007; Miranda et al. 2009; Moussa et al. 2010; Aftab et al., 2012; Elmanama et al., 2013; Iyer et al., 2013; Anihouvi et al., 2013; Adeyanju and Ishola, 2014). *Salmonella* is regarded as a zero tolerance organism in foods and should not be present in food, thus the *Salmonella* testing of food samples is mandatory (Chattopadhyay et al., 2013). However, *Salmonella* is ubiquitous in nature, and can be found in the digestive tracts of different animals, poultry products, eggs, milk products and seafood. Raw chicken meat is known to be the major source for *Salmonella* food poisoning (Chen et al., 2008).

E. coli has been implicated as an agent of diarrheal disease. Diarrheagenic strains of *E. coli* can be divided into five main categories on the basis of distinct epidemiological and clinical features, specific virulence factors, and association with certain serotypes: EAEC, EHEC, EIEC,

EPEC, DAEC and ETEC (Nguyen et al., 2005; Gómez-Duarte et al., 2006). The prevalence of diarrheagenic *E. coli* was 22.5% among children suffering from diarrhea (Nguyen et al., 2005; Hien et al., 2007). Results of this research showed that 95% (38/40) of meat samples were contaminated with *E. coli*, and 89.5% (34/38) of these meat samples contaminated with *E. coli* were diarrheagenic. The prevalence of *E. coli* in different food ranged from 9.1% to 100% (Zhao et al 2001; Rivera-Betancourt et al., 2004; Cohen et al. 2007; Lee *et al.* 2009; Saikia and Joshi 2010; Ukut et al. 2010; Biswas et al., 2010; Abdellah et al., 2013; Iyer et al., 2013; Adeyanju and Ishola, 2014). High prevalence of *E. coli* and/or diarrheagenic *E. coli* can be explained due to that *E. coli* isolates are part of the normal enteric flora in these animals. A total of 39 pathogenic *E. coli* isolates from the three meat types (fresh beef, poultry and pork) were categorized into three virulence groups, comprise of ETEC (43.6%), EHEC (35.9%), and EPEC (20.5%) (Lee *et al.* 2009).

The prevalence of foodborne pathogens from food samples differs among studies. This could be due in part to several factors such as differences in the reservoir in the various countries or ecological origin of strains, the sensitivity of detection methods, detected genes and number of samples, and type of samples analyzed (whole birds versus steaks; fresh versus frozen), time of sample collection and type of storage (Zhao et al.2001; Adwan *et al.*, 2005). The poor hygiene and sanitation prevailing in the abattoirs as well as the shops, unwashed carcasses, transportation and display at butcher shops encourage microbial contaminations and growth.

The higher microbial load in the shops further enhances the chances of early meat spoilage (Bhandare *et al*, 2007).

Several factors could have close relationship with the heavy bacteriological load in different meat types studied in this research. The main factor is the climate surrounded the studied region. In the current study Jenin government is considered the lowest city from the sea level in the north of Palestine, which considered the main reason for the high temperature and humidity in the region, where the suitable condition for high microbial growth. In addition, the direct exposure of meat to the open air in most shops are studied is considered another important factor that leads to the high microbial load.

Therefore, it is recommended to establish a permanent program for surveillance of microbial contamination with all food-borne pathogens. This may generate and provide data with statistical and epidemiological value. These data can be used for estimating the exposure of consumers to foodborne pathogens and evaluating the effects of control measures on the contamination of food. It is well known that contamination of food items that are usually consumed after cooking represents a low threat from a public health point of view; however, more attention should be paid to the contamination of ready-to-eat products. Consumption of undercooked meat products and cross-contamination during food handling and preparation must be avoided to ensure food safety at home and in the food service industry.

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جامعة النجاح الوطنية

كلية الدراسات العليا

استخدام تقنية تفاعلات البلمرة المتسلسلة للكشف عن مسببات الامراض البكتيرية
في منتجات اللحوم في محافظة جنين - فلسطين

اعداد

براء رضوان محمود القرم

اشراف

د. غالب عدوان

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في برنامج العلوم
الحياتية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، في نابلس - فلسطين.

2014

ب

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الملخص

تحدث الأمراض المنقولة عن طريق الأغذية في جميع أنحاء العالم، من خلال استهلاك اللحوم الملوثة. وقد أجريت هذه الدراسة للبحث في انتشار كل من المكورات العنقودية الذهبية المنتجة للسموم المعوية، السالمونيلا والإشريكية القولونية بأنواعها الممرضة في 40 عينة من اللحوم الطازجة (ن = 35) والمجمدة (ن = 5) والتي اشتملت على لحوم البقر والديك الرومي والدجاج، باستخدام تقنية تفاعلات البلمرة المتسلسلة المركبة. تم شراء عينات اللحوم من الأسواق المحلية في محافظة جنين، فلسطين. وأظهرت نتائج هذا البحث أن العدد الكلي للبكتيريا الهوائية متوسطة الحرارة تراوحت بين $4.3 \log_{10}$ إلى $5.7 \text{ cfu} \log_{10}$ /غرام لعينات اللحوم المجمدة و $6.95 \log_{10}$ إلى $7.78 \text{ cfu} \log_{10}$ /غرام لعينات اللحوم الطازجة. وأظهرت النتائج أن معدل انتشار بكتيريا المكورة العنقودية الذهبية، السالمونيلا والإشريكية القولونية كان 30%، 25% و 95% على التوالي. وقد وجد أن 75% من البكتيريا العنقودية الذهبية كانت منتجة للسموم المعوية. وجدت عينتين من غير البكتيريا العنقودية الذهبية (*FemA* -) منتجة للسموم المعوية، احداها sec^+ والآخرى كانت see^+ . وأظهرت هذه النتائج أن 89.5% (38/34) من عينات اللحوم الملوثة بالبكتيريا الاشريكية القولونية ينتمي إلى كل من الانواع الممرضة التالية ، الاشريكية القولونية المسببة للنزف المعوي (EHEC)، الاشريكية القولونية العوية الممرضة (EPEC)، الاشريكية القولونية المعوية المتحوصلة (EAEC)، الاشريكية القولونية السمية المعوية (ETEC) والاشريكية القولونية المنتشرة (DAEC).و قد اظهرت الدراسة ايضا ان ما مجموعه 21% (38/8) من عينات اللحوم الملوثة بالاشريكية القولونية احتوت تلوت مشترك بهذه الانواع الممرضة.

ج

فمن المستحسن وضع برنامج دائم لمراقبة التلوث الميكروبي لكل مسببات الأمراض المنقولة
بالغذاء.

هذا قد يسعى بتوليد وتوفير بيانات ذو قيمة احصائية ووبائية. ويمكن استخدامها لتقدير
التعرض لمسببات الأمراض المنقولة عن طريق الأغذية للمستهلكين وتقييم آثار تدابير الرقابة على
تلوث الأغذية.