Faculty of Pharmacy Nursing and Health Professions
Master Program in Clinical Laboratory Science

Level of microbial contamination in slaughterhouses compared to traditional chicken slaughter stores

مستوى التلوث الجرثومي في المسالخ مقارنة بمتاجر ذبح الدواجن التقليدية

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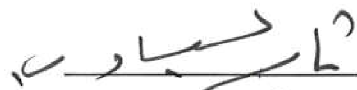
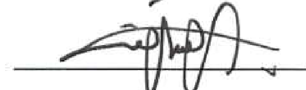
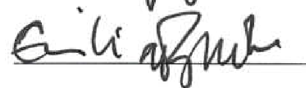
Dr. Tamer Essawi/ Supervisor	
Dr. Ibrahim Al-Zuheir/ External examiner	
Dr. Emilia Rappocciolo/ Internal examiner	

Climbing to the top and passing all the obstacles to reach it is not only an individual effort but an ongoing effort, support and encouragement from all the lovers around you.

I dedicate this dissertation to my precious parents, who have taught me to always look towards the summit and provide me with all the tools I need to reach.

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Husam Sabe Alsayed

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Level of microbial contamination in slaughterhouses compared to traditional chicken slaughter stores

مستوى التلوث الجرثومي في المسالخ مقارنة بمتاجر ذبح الدواجن التقليدية

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

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Ethical Consideration

Commitment at Birzeit University laws and ethics of scientific research will be taken strictly in this study. Appropriate permission and references will be ensured for the usage of any given data, confidentiality will be maintained strictly to ensure the privacy of any given data, disclosure of identity will not be exhibited. usage of any data from any source will be acknowledged with appropriate reference. Hence, the ethical aspect of the research will be followed strictly in this research.

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List of Abbreviation

Symbol	Abbreviation
CFU	Colony-forming units
TVC	Total viable count
µg	Microgram
µl	Micro liter
bp	Base pair
DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
NaCl	Sodium Chloride
psi	Pounds per square inch
dNTP	Deoxynucleotide
Taq	*Taq* polymerase
g	Gram
H_2S	Hydrogen sulfide
hrs.	Hours
Min	Minute
Sec	Second
N	Number
M	Molar
Fig	Figure
P	P value
umol	Micro mol
mg	Milligram
ml	Milliliter
ºC	Degree Celsius
PCBS	Palestinian Central Bureau of Statistics
SPSS	Statistical Package for the Social Sciences
PSI	Palestinian Standards Institution
GMP	good manufacturing practice
3rd party	Third-party body

PCR	Polymerase chain reaction
pH	a scale of acidity from 0 to 14
Rpm	Round Per Minute
Spp.	Species
Seq	Sequence
Ppm	Part per million
S. aureus	*Staphylococcus aureus*
L. mono	*Listeria. Monocytogenes*
E. coli	*Escherichia coli*
C jejuni	*Campylobacter jejuni*
Cl. perfrin	*Clostridium perfringens*
NA	Nutrient Agar
MSA	Mannitol Salt Agar Base
SS agar	*Salmonella Shigella* agar
U.S.A	United States of America
WHO	World Health Organization
COP	Continuous online processing
ASC	Acidified sodium chlorite
USFDA	U.S. Food and Drug Administration
IOBW	inside outside- bird-washer

Abstract

Poultry meat processing is crucial to determine the quality of the final product. Poultry meat can be contaminated with pathogenic microorganisms responsible for cases of human infection and food poisoning. Humans can become exposed to pathogens originating from poultry meat during different stages of processing. There are two ways of poultry meat processing in Palestine: the first and most popular one is the small-scale slaughtering store, the second and more recent is the modern large-scale slaughterhouses. The objective of this study is to compare the small and large scale slaughtering methods in terms of bacterial contamination as total viable count, and to determine the risk factors of bacterial contamination in the different stages of the production process in both methods. 90 swap samples of inside-outside surfaces of final products distributed on 10 locations (5 small and 5 large scale slaughterhouses) from three targeted cities covering West Bank in Palestine were taken and tested for the presence of *Salmonella spp., Campylobacter spp., Clostridium perfringens, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus* and *Proteus spp.* by culturing on selective medias and PCR. In addition, a questionnaire was performed to correlate the risk factors of different processing practices with the laboratory findings. Laboratory results and questionnaires were analyzed by SPSS. The results showed that large scale slaughterhouses was not significantly lower in term of total viable count than small scale slaughterhouses, furthermore *Salmonella spp., Clostridium perfringens, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus and Proteus spp.*were present in both methods ($P > 0.05$). In contrast, *Campylobacter spp*. was significantly higher in the small scale than in the large scale slaughterhouses ($P < 0.05$). conducting of legal registration issues, establishment of modernized infrastructure and modern slaughtering processes decrease bacterial contamination and enhance meat quality. Slaughterhouse grading system should be designed and monitored by competent authorities. Presence of diagnostic tools in slaughterhouse own laboratory and data references for all the procedures, and presence of veterinarian and health inspection all the time for health professions with help of quality control manager will lead to the best results with less possible bacterial contamination.

ملخص الدراسة

تعتبر العمليات الحيوية المتبعة خلال معالجة لحوم الدواجن أمرًا مهمًا في جودة المنتج النهائي. حيث بالإمكان حدوث أي تلوث لهذه اللحوم من خلال الكائنات الحية الدقيقة المسببة للأمراض المسؤولة عن حالات التسمم الغذائي البشري، الناجمة عن تعرض الإنسان لها خلال مراحل المعالجة المختلفة. هناك طريقتان لمعالجة لحوم الدواجن في فلسطين ؛ الطريقة الأولى والأكثر شعبية هي الطريقة التقليدية لذبح الدواجن من خلال متاجر الذبح الصغيرة ذات الطاقة الإنتاجية القليلة، أما الطريقة الثانية و الحديثة و التي ظهرت مؤخراً هي مسالخ الدواجن الحديثة ذات الطاقة الإنتاجية العالية. تهدف هذه الدراسة إلى المقارنة بين طرق الذبح الصغيرة ذات الطابع التقليدي والكبيرة ذات الطابع الحديث، من حيث نوع التلوث البكتيري الموجود والعدد البكتيري الكلي في كل منهما، إضافة إلى تحديد عوامل الخطر المرتبطة بحدوث التلوث البكتيري خلال مراحل الإنتاج المختلفة في كلا الطريقتين. تم القيام بهذه الدراسة عن طريق أخذ وفحص 90 عينة من الأسطح الداخلية والخارجية لمنتجات الدواجن النهائية والموزعة على 10 مواقع (5 مسالخ صغيرة و 5 مسالخ كبيرة الحجم) من ثلاث مدن تم استهدفها لتغطي الضفة الغربية في فلسطين وتم اختبارها لوجود : *Salmonella spp, Campylobacter spp, Clostridium perfringens, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Proteus spp*

من خلال فحص جميع العينات و زراعتها على بيئات النمو الإنتقائية و من خلال PCR ، إضافة إلى ذلك، تم إجراء استبيان مصمم بشكل دقيق لربط عوامل الخطر المرتبطة بحدوث التلوث البكتيري مع النتائج المخبرية. تم تحليل نتائج المختبر والاستبيانات المملوءة بالكامل، وقد أظهرت النتائج أنه لا توجد فروق ذات دلالات إحصائية ($P> 0.05$) بين كلا طريقتي الذبح من حيث العدد البكتيري الكلي و من حيث وجود

Salmonella spp, Clostridium perfringens, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus spp*

في المقابل, كان وجود ال *Campylobacter spp* في متاجر الذبح الصغيرة أعلى بكثير من مسالخ الدواجن الحديثة، و كان الفرق بينهما ذات دلالة إحصائية ($P <0.05$).

وقد خلصت الدراسة إلى أن إنجاز التراخيص القانونية المتبعة لإتمام عمليات التسجيل الرسمي للمنشآت و الحصول عليها، وإنشاء بنية أساسية صحيحة مبنية بشكل علمي و حديث، إضافة إلى اتباع النظام الحديث لعمليات الذبح يقلل من التلوث الجرثومي ويعزز جودة اللحوم و المنتجات النهائية قبل وصولها للمستهلك. و خلصت أيضا إلى أن تصميم نظام تقييمي للمسالخ خاضع لرقابة الجهات المختصة، و وجود الأدوات التشخيصية المخبرية الخاصة بالمسالخ إضافة لسجلات البيانات المتصلة لجميع الإجراءات السارية، و وجود طبيب بيطري يشرف على جميع إجراءات الفحص الصحي بشكل دائم و بمساعدة ضابط الجودة يؤدي و بشكل مباشر لأفضل النتائج و لتقليل التلوث البكتيري لأقصى حد ممكن.

Chapter I: Introduction

Poultry is one of the most important sources of white meat in the world after marine fish, due to its high protein content and reasonable price for most community classes. It contains about 27 grams of protein per 100 grams of chicken, as well as high nutritional value and less damage to health (Marangoni, Corsello et al. 2015).

Despite the deterioration of the agricultural sector in Palestine as a result of many "Israeli" policies, the poultry industry in Palestine has increased rapidly throughout the past years, and it occupies a high economical important place in the local market (Palestinian central bureau of statistics 2014). The poultry sector has played a major role in maintaining its position as a major sector contributing to the Palestinian economy, it contributes 40% to 50% of livestock production and 12% to 15% of agricultural production in Palestine (Palestinian central bureau of statistics 2014)

Poultry farming is generally divided into two main sections: raising chickens for meat production (broiler chickens) and raising chickens for the production of eggs (layer chickens). According to the Palestinian Central Bureau of Statistics, the number of broiler chicks produced in Palestine was about 53 million chicks in 2017. Slaughtered birds in slaughterhouses operating in Palestine reached about 7 million birds in 2017, of which 95% were in the West Bank (Palestinian central bureau of statistics 2018). In addition to the heavy production of broiler chickens, and the increase of the number of farms and chicken herds, chicken are prepared and processed to reach the consumer through two types of slaughtering centers: small-scale slaughter stores, large-scale slaughterhouses.

The number of large slaughterhouses in Palestine is 10 slaughterhouses, of which 9 slaughterhouses are located in the West Bank, of these three are not registered in government records, while only 1 large slaughterhouse has been registered in the Gaza Strip (Palestinian central bureau of statistics 2014).

Unfortunately, there are no records or statistics related to the number of small slaughterhouses in Palestine, either through the Palestinian Central Bureau of Statistics or the records of the Palestinian Ministry of Agriculture (Palestinian central bureau of statistics 2014). Both methods, either the small slaughterhouse or the large slaughterhouse almost follow the same major processing steps for poultry production, but they differ in the delivery of each step.

In spite of the absence of official health control on the source of poultry, its state of health, the method of treatment and even the storage conditions in the small slaughterhouse, they are still the most popular and preferred in the Palestinian local market. Large-scale slaughtering depends on scientific and systematic stages, followed by several major steps. The main stages being: slaughtering, bleeding, scalding, de-feathering, evisceration, washing, chilling, and finally classification (Tsola, Drosinos et al. 2008).

Slaughtering is the first stage of the humane slaughter in large sclae slaughterhouses by using electrical stunning so that the bird loses consciousness before killing. Within seconds after stunning, the shackle conveyor moves and the poultry will be transported to the place where they will be killed manually according to Islamic law (Welty 2007). After slaughtering and cutting the neck, the bird is allowed to bleed for approximately 220 seconds, then the scalding stage begins at 52-57 ° C for about 150 seconds, it’s important to loosen and soften the feathers without causing significant damage to the outer skin layers. After scalding, the carcass is ready for the

feather removal stage (defeathering) after scalding finishes, using a feather removal machine (plucking machine) which consists of a series of rotating rows of elastic and polygonal fingers to pull down the soften feathers (Tsola, Drosinos et al. 2008). Following defeathering, the birds are eviscerated by removing the edible and non-edible viscera from the carcass. In this stage the cavities of the carcasses are opened by making a cut from the posterior tip of the breast bone to the cloaca (anus), the viscera are scooped out, and the edible viscera or "giblets" (heart, liver, and gizzard) are harvested from the extracted viscera, trimmed of adhering tissues, and washed with water; the carcass is released from the evisceration step after washing and goes toward the chilling stage (Tsola, Drosinos et al. 2008). Carcass chilling at a temperature of (0-4) °C or less is achieved as soon as possible after evisceration. The primary objective of chilling poultry is the reduction of microbial growth to a level that will maximize both food safety and time available for marketing. The two most common methods of poultry chilling are water and air chilling. After all these stages, carcasses are classifieds according to weight and packaged for storage and shipping (Tsola, Drosinos et al. 2008).

Small scale slaughter stores follow a system that is similar to the large scale slaughter plants, but that is less accurate in the delivery of the processing steps. Processing in small scale slaughtering stores is done manually for the whole process, except in the step of plucking. For the first stage, the store workers slaughter each bird separately without stunning, leave it for a few seconds to bleed, and then put it for another few seconds in warm water tank, similar to a scalding step. Then, the carcass moves to a plucking machine which accommodates to five to six birds for defeathering. After defeathering the birds are eviscerated by removing edible and inedible viscera from the carcass, the carcass is washed with tap water and delivered to the customer directly without chilling or classification.

Like any type of meat, poultry meat is exposed to bacterial contamination from microorganisms through many sources: (1) Raising of the broilers chickens on litter floors, may lead to contamination of poultry with spoilage microorganisms and also with human pathogens, some of the poultry are healthy carriers of pathogens and they are not excluded from the farm or slaughter during antemortem inspection because they are not showing any symptoms of bacterial infection (Keener, Bashor et al. 2004). (2) The transportation system also affects the extent of bacterial contamination due to stress during transport, and due to excretion patterns of birds carrying pathogens. The slaughterhouse environment is considered a main source of bacterial contamination, including live poultries, equipment, and working staff in addition to any defect in the process of slaughtering or handling that may lead to cross-contamination with fecal materials in the digestive system of birds and surviving of these pathogens (Cox and Pavic 2010).

The advantage of modernization is in decrease of contamination due to less human involvement, but the disadvantage is that contamination might increase due to inadequate cleaning of equipment. Producing a product free from bacterial contamination is one of the most important criteria for the goodness and quality of the final product (Tsola, Drosinos et al. 2008). The most important task in the production of human food is to prevent the product from being poisonous. This is done through several checks and controls; the most important factor however is reducing the total bacterial count to the lower limit, thus extending the shelf life of the product. Reducing the risk factors in the production chain by producing a better final product will allow for better quality production of poultry meat, either for export criteria or for the domestic market consumption (Owens, Sams et al. 2000).

A quantitative evaluation of the level of bacterial contamination, identification, and quantification of the most important zoonotic bacteria in both small-scale slaughter stores and large-scale slaughterhouses is required. These data in relation to the type of control and monitoring of bacteria during slaughtering process line in both methods will provide accurate information on the effectiveness of the system and the safety of the final poultry product for human consumption.

1.2 Aim of study:

The aim of this study is to compare large scale slaughterhouses and small scale slaughter stores production process in term of poultry meat bacterial contamination.

1.3 Objectives of study:

- To determine bacterial total viable count (TVC) in the final poultry meat products obtained from the local market of both processing method.
- To investigate the presence of: *Salmonella spp., Campylobacter spp., Clostridium perfringens, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus* and *Proteus spp.* in the final product of small and large scale slaughtering stores.
- To evaluate the impact of processing in small and modern large-scale slaughter plants (slaughtering, bleeding, scalding, de-feathering, evisceration, washing, and chilling) on the total viable count.
- To determine the risk factors of bacterial contamination of the production process in both methods.

1.4 Statement of problem:

The goodness and quality of the final product determine its shelf life, low-quality poultry meat reduces the shelf life of the product and creating a health threat to the consumers. In recent times there have been many complaints by citizens about the quality of poultry meat, that was also accompanied with the arrest of rotten chicken meat shipments by competent authorities, in addition to the arrest of smuggled shipments of unknown sources of live chickens. Moreover, there are no sufficient control and supervision on the large scale slaughterhouses and small scale slaughter stores by competent authorities. This created a worried and uneasy feeling about the real quality and goodness of poultry meat and their threat to human being health.

"Level of microbial contamination in slaughterhouses compared to traditional chicken slaughter stores" is the first study in Palestine concerned with the level and type of bacterial contamination in large and small slaughterhouses. The outcome of this study would be used as a guide for the Palestinian customer and decision-makers for producing healthy as well as safe products.

Chapter II: Literature Review

2.1 Poultry production in Palestine

Poultry occupies an important position as a source of meat and egg production, both of which have excellent nutritional value in human food (Barroeta 2007). Chicken is at the forefront of all poultry species; for its ability to convert food into an animal protein with high nutritional value (Barroeta 2007). In Palestine, poultry is reared in numbers appropriate to the conditions of the farms; they may be small numbers in the houses or may reach several thousand, depending on the possibilities and conditions of the breeder (Palestinian central bureau of statistics 2014). Broiler chicken has a high genetic efficiency to convert feed material into meat. To maximize this advantage, the environmental conditions surrounding and feedings of chicken must be improved to make it similar to the optimal conditions of chicken life. This increase the profitablity.

The utilization of poultry meat as a source of white meat is increasing tremendously worldwide (Scanes 2007). As poultry meat is recognized for its low energy and high nutrient value. It is considered as a good source of high biological protein value (20-22%), as well as provides minerals and vitamins of high bioavailability in lower quantities than red meats (Table 1) (Barroeta 2007).

Besides its low prices compared to the red meat, poultry meat is a part of traditional Palestinian food as well being used as gifts by rural people to strengthen social relationships (Palestinian news and info agent 2019).

Table 1: Nutritive value of poultry meat, per 100g of the edible portion

	Whole	Breast		Whole	Breast
Water (g)	70.3	75.4	Vitamins		
Energy (kcal)	167	112	Vitamin B_1 (mg)	0.1	0,1
Protein (g)	20.0	21.8	Vitamin B_2 (mg)	0.15	0.15
Total fat (g)	9.7	2.8	Niacin eq. (mg)	10.4	14
SFA (g)	2.6	0.76	Vitamin B_6 (mg)	0.3	0.42
MUFA (g)	4.4	1.3	Biotin (µg)	2.0	2.0
PUFA (g)	1.8	0.52	Folic acid (µg)	10	12
PUFA/SFA	0.69	0.69	Vitamin B_{12} (µg)	0.4	0.4
Cholesterol (mg)	110	69	Vitamin C (mg)	—	—
Minerals			Vit. A: Eq. Retinol (µg)	9	16
Calcium (mg)	13	14	Vitamin D (µg)	0.2	0.2
Iron (mg)	1.1	1.0	Vitamin E (mg)	0.2	0.29
Iodine (µg)	0.4	0.4	Vitamin K (µg)	—	—
Magnesium (mg)	22	23			
Zinc (mg)	1	0.7			
Selenium (µg)	6	7			
Sodium (mg)	64	81			
Potassium (mg)	248	320			
Phosphorus (mg)	147	173			

Adapted from (Barroeta 2007)

2.1.1 Poultry hold in Palestine

Production and consumption of poultry meat and poultry meat products show an upward trend globally (Scanes 2007). Poultry farming is now called the "poultry industry". It is handled during the service phases, manually or automatically with full mechanization. Poultry farming is mainly divided into two parts: 1- Breeding chickens to produce meat (broiler chickens), 2- Breeding chickens for egg production (laying hens). In Palestinian agriculture, the poultry production sector is one of the most important sectors, its importance comes from the increasing investments in the livestock and poultry meat processing plants (Palestinian news and info agent 2019). During the last decade the number of both layers and broilers has increased dramatically (Palestinian central bureau of statistics 2014), it occupies a high economical important place in the local market. Breeding chickens for meat production is done by accelerating growth from the earliest stages of the cycle, with the tendency to fatten them to the maximum possible weight, and in the shortest possible period of time. Appropriate and rapidly growing are the most important factors that must be available for the success of broiler chicks.

It is estimated that the average consumption of broiler chickens in the West Bank is 16 chickens per capita per year; in the Gaza Strip, 12 chickens per capita per year (Palestinian news and info agent 2019). The normal mortality rate in broiler farms reached 10%; indicating the need to raise production to 40-44 million carnivorous chicks per year in the West Bank; and 21 million chickens in the Gaza Strip (Palestinian news and info agent 2019). There are 17 chicks' hatcheries in the West Bank. The production capacity of these hatcheries is 96 million hatching eggs annually; however, the annual production capacity in these hatcheries ranges between 52-57%. According to the statistics of the Palestinian poultry sector, the production capacity of broiler farms in the West Bank promises a surplus of broiler production (Palestinian central bureau of statistics 2014). According to the records of the Palestinian Ministry of Agriculture published by PCBs; the number of bred broiler chickens gradually increased between 2015 and 2017 to reach about 53 million birds in 2017 (Palestinian central bureau of statistics 2018). In spite of this, the records of Palestinian Central Bureau of Statistics (PCBS) for the slaughtered chickens were significantly different.

2.2 Poultry slaughtering

To achieve the profitability in poultry meat products industry, all the processes in the poultry meat production must be standardized to produce a good quality of the final product. This also must consider animal welfare and environment concerns. Poultry processing includes event from the farm, slaughtering, and processing steps.

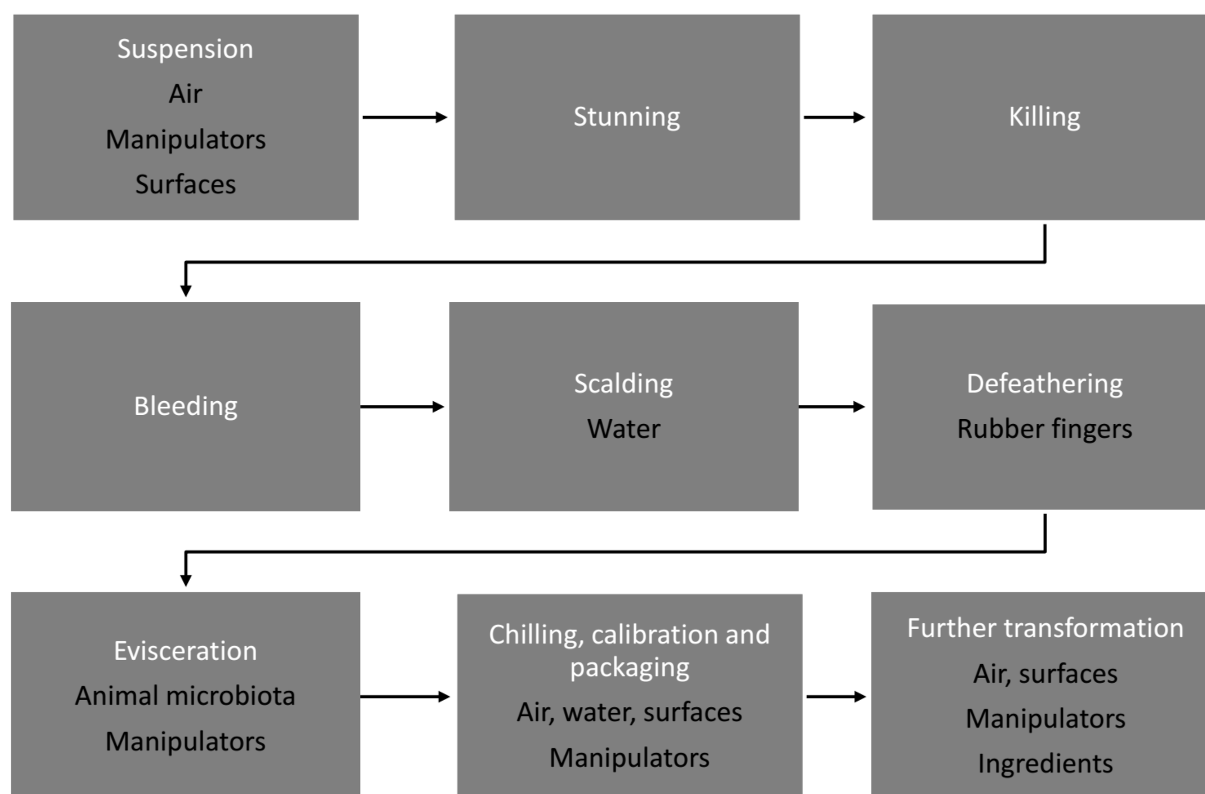

Figure 1: Slaughtering process (Rouger, Tresse et al. 2017)

Poultry meat and poultry products' marketing structures have not been well studied in Palestine. The market outlets or channels available to producers are diverse. The major channels through which producers/farmers sell their products in Palestinian markets (as one of the developing) are either direct-sold to consumers and/or to small retails in the market, or to new large markets (Kondombo 2005). However, the smallholder farmers do have little knowledge of the correct hygienic conditions and why poultry product quality fluctuates. Thus, most farmers sell chickens within their vicinity. There are three ways of poultry meat processing; the first and most popular one is the small scale slaughtering, and recently the modern large-scale slaughter plants. The on-farm slaughtering is not practical in Palestine (Table 2).

Table 2: Comparison between small and large scale slaughterhouses in Palestine

	Small scale slaughterhouses	large scale slaughterhouses
Size	50 - 250 m^2	500 - 3000 m^2
Equipment	Manual \ except for scalding	Fully automated
Cost	Less than 30000 $	More than 500000 $
Labor	Less than 10 employees	40 - 80 employees
Production Capacity	300 - 1000 birds per day	5000 - 30000 birds per day
Operation	Year-round - processed daily	Year-round - processed daily
Marketing	Products sold fresh, whole birds	Products sold fresh and frozen, whole birds and parts

The number of slaughtered chickens on the large scale slaughterhouses in 2017 was about 7 million. This constitutes only 13% of the bred chickens (Palestinian central bureau of statistics 2014). There are 9 large scale slaughterhouses in West Bank according to PCBS, 6 of them are registered in governmental records while the remaining 3 are not. According to PCBS, the ministry of agriculture and all relevant governmental agencies, there was not any records or statistics about the number of small scale slaughter stores in the west bank (Palestinian central bureau of statistics 2014). By personal estimation and data collection based on the population of the West Bank, considering 1 store per 1500 persons, there should be more than 2000 small scale slaughter stores in the West Bank. According to these records, there is a huge gap in the number of slaughtered birds compared to bred chickens; about 85% of slaughtered birds were out of statistical records, supervision and inspection of governmental authorities.

The slaughtering process differs between small and large scale slaughterhouses; these differences will be mentioned at each slaughtering process step. In small scale slaughterhouses, the birds are produced in small numbers and they are marketed mostly as fresh, un-chilled meat. For these reasons, poultry may need to be slaughtered nearer or in the market. More information about

each stage and the differences between large and small slaughterhouses is discussed below. The figure illustrates a schematic processing

2.3 Slaughtering Procedures

During the production and management of poultry, both the pre-slaughter and the post-slaughter factors exert important effects on meat quality, composition, and development. For this, the events that occur both before and after the slaughtering or the processing of the poultry influence the final meat quality. The main processing steps of poultry meat consist of slaughtering, scalding, de-feathering, evisceration, washing, chilling and packaging. After slaughtering and bleeding of chickens, the carcass is scalded at hot water for a specific time, followed by de-feathered by before evisceration (Tsola, Drosinos et al. 2008), finally washed with water before final chilling and packaging.

2.3.1 Pre-slaughter handling

At the end of rearing period, before the transport, the birds are taken off feed and water for overnight. This allows evacuation of the digestive tract and reduces the contamination during processing. At night the birds are caught by specially trained crews and placed into plastic transport cages. The birds are then transported to the slaughterhouse, no differences occur in this step between the small and large scale slaughtering process.

2.3.2 Unloading

The catching of the birds on the farm and the transfer to the slaughterhouse can be a stressful process. Stress can negatively affect meat quality and interfere with the processing. Improving the pre-slaughter processes is important to minimize this effect (Ali, Kang et al. 2008). In small scale slaughterhouses, the birds are kept in the cages at the selling point and the slaughter

process start at the customer request. Food and water are available to the birds the whole time. In contrast; at the large scale slaughterhouses, the birds are removed from the cages and transferred to continuously moving shackles where they are suspended by both legs. The receiving room is dark and contains red light to keep the birds calm (Sams 2000).

2.3.3. Stunning

Stunning is the first step in humane slaughter. This renders the birds unconscious prior to the killing. In the large scale slaughterhouses, several methods have been developed to accomplish this goal. The most common and one of the simplest is electric shock. While hanging by their feet, the heads of the birds contact a saline solution (approximately 1% NaCl) that is charged so that an electrical current flows through the bird to the shackle line which serves as the earth (Ali, Kang et al. 2008). Electrical stunning produces about 60 to 90 sec of unconsciousness during which the bird can't move or feel any pain. Immediately after contact, the legs are extended and the wings are bound tight against the body. This allows the birds to relax and the neck to arch to perform a humane slaughtering (Owens, Sams et al. 2000). No stunning is carried out in the small-scale slaughterhouses.

2.3.4. Killing

In large scale slaughterhouses, within seconds after stunning the shackle line moves the bird to the killing site. A trained employee grabs the wattles and lower neck skin to hold and guide the head, and use a sharp knife to cut the jugular veins and carotid arteries on one or both sides of the neck of the bird. The birds are let to bleed for a fixed amount of time (60-90 seconds) (Owens, Sams et al. 2000). In the small scale slaughterhouse, the employee grabs the chicken from the wings and bends the neck, using a sharp knife to cut the jugular veins and carotid

arteries. No fixed amount of time for bleeding is allowed, bidrs are let to bleed until completely at rest.

2.3.5. Scalding Feather removal

In the large scale slaughterhouses, following bleeding, the bird carcass is moved along the belt for removing of the feathers. Feathers are difficult to remove in their native condition due to their attachment in the follicles. To loosen them, the carcasses are submersed in multistage tanks of hot water which serves to denature the protein structures holding the feathers (Owens, Sams et al. 2000). The tanks contain hot water which enhances the softening of the skin to facilitate the removal of the feathers. In the large scale slaughterhouse, the temperature of the water is carefully controlled. The time and temperature combination is important to perform proper de-feathering without causing damage to the outer skin layers. In the small scale a slaughterhouse, the carcass is immersed in one single tank contains boiling water for the time estimated by the employee with continuous moving till losing the feather (Fig 2).

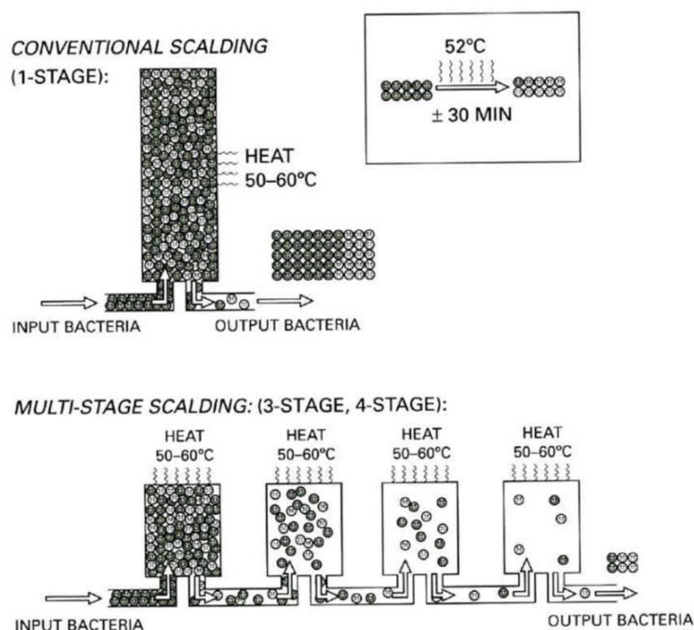

Figure 2: Comparison of scalding in small and large scale slaughterhouses. **(Davies, Board et al. 1998)**

When the skin becomes loosened, the outer skin layer is removed by the abrasion of the mechanical Picking machines. Picking machines consist of rotating clusters of flexible, rubber fingers rotating rapidly, the fingers rub against the carcass and removes out the loosened feathers. By combining a series of these rotating clusters of fingers, each directed at a different region of the carcass, the whole carcass is picked Pin feathers are small feathers that protrude from the skin and can't be removed with the machines. This requires manual attention (Owens, Sams et al. 2000). Usually, carcasses are briefly passed through a flame to burn off the hair-like feathers on the skin.

Before leaving the picking area, the heads are pulled and the feathers, blood, feet, and inedible viscera (called "offal") are sent away from the processing area (either in-plant or at a different location), where these materials are ground and cooked into poultry fat and byproduct meal for other industries (Owens, Sams et al. 2000).

The carcass is then moved from the killing line to the evisceration site; this can be a site of bacterial cross-contamination, as one employee handles many birds. The separation between the live and dead areas is important to reduce contamination of the relatively cleaner evisceration room. There is no separation between the different processes in the small scale slaughterhouses.

2.3.6. Evisceration

Evisceration is the removal of the viscera from the carcass. In broilers, evisceration has three basic objectives: (1) the body cavity is opened by making a cut from the posterior tip of the breastbone to the cloaca; (2) the viscera (primarily the gastrointestinal tract and associated organs, reproductive tract, and lungs) is removed out; and (3) the other organs (haert, liver, and gizzard) are harvested from the extracted viscera, trimmed of adhering tissues, and washed with water. In small scale slaughterhouses, the evisceration is performed manually by making a circular cut around the vent to draw out the visceral organs (intestines, esophagus, spleen, reproductive organs). Instead of using shackles, small scale slaughterhouses processors usually eviscerate on a at surface and the visceral organs are drawn manually by hand. In contrast, large scale slaughterhouses use automated machines that remove out the guts; high-speed lines eviscerate 2,000 to 8,000 birds per hour (Owens, Sams et al. 2000). The process is highly coordinated with automated operations.

2.3.7.Chilling

Water immersion chilling, air chilling and water spin chilling are the three common chilling methods for poultry products. The chilling method is chosen depending on the types of final products. Immersion chilling with chlorinated water is applied mainly to freeze carcasses while water spin chilling and air chilling are used for fresh poultry meat or poultry meat processing planets (Davies, Board et al. 1998). The chilling process of carcasses aims at inhibiting the

growth of contaminating microorganisms. The usage of chlorinated water has an impact on the final viable counts of coliform and other bacteria (Owens, Sams et al. 2000). The products of small scale slaughterhouses are washed with tap water and direct-sold to consumers and/or to small retails in the market without a chilling process.

2.3.8. Packaging

The growth of pathogens and spoilage microorganisms is the main concern during packaging. Therefore, the materials and methods used to pack poultry meat depend on the type of products and the slaughtering process. In the large scale slaughterhouses, the packaging methods are vacuum packaging, carbon dioxide flushing packaging, and modified atmosphere packaging (MAP) (Lee, Sebranek et al. 1996). In small scale slaughterhouses, the traditional wrapping packaging with plastic bags is performed. No data will be written on the product. The product is directly sold or distributed to near markets.

2.4. Poultry Meat Microbial contamination

Poultry meat can be contaminated during the slaughtering, processing, storage, handling and packaging steps. This occurs by contact of the carcass with bird body parts which contain a high bacterial load (e.g., feathers, feet, intestinal contents), or due to contact with contaminated equipment, or employees during manipulation of the meat (Conner, Davis et al. 2000). Poultry meat provides an excellent medium for the growth of microorganisms. The principal contaminating bacteria that can be found on poultry include *Pseudomonas, Staphylococcus, Micrococcus, Acinetobacter*, and *Moraxella* (Bryan and Doyle 1995). In addition, poultry meat supports the growth of certain pathogenic bacteria, such as *Salmonella* (Chia, Goulter et al. 2009). Meat borne zoonotic diseases such as *Salmonella, Campylobacter, and E. coli* are

reported in poultry products. In addition, food poisoning by *Clostridium, Staphylococcus*, are a major problems that results from consumers eating contaminated meat. The microbial load assessment and bacterial type are critical measures to assure meat quality.

2.4.1. *Salmonella*

Salmonellosis is a meat borne zoonotic disease in humans, causing worldwide problems (Flint, Van Duynhoven et al. 2005). *Salmonella* is a member of the family *Enterobacteriaceae*. They are enteric bacteria, Gram-negative, facultative anaerobic rod-shaped bacilli. The genus Salmonella consists of two species (*Salmonella enterica* and *Salmonella bongori*) and other six subspecies (Graziani, Losasso et al. 2017). The contamination of poultry meat with *Salmonella* is associated with gastroenteritis in humans. Poultry intestines tested by culture methods using selective media and PCR technique in Hebrew University of Jerusalem showed the presence of *Clostridium, Salmonella and E. coli*. and others (Amit-Romach, Sklan et al. 2004). Poultry meat can be contaminated throughout any step in the production chain.

2.4.2. *Campylobacter*

Campylobacter also is one of the most common bacterial causes of diarrhea worldwide. The most-reported species of *Campylobacter* causing human illness is *Campylobacter jejuni*, but other *Campylobacter* species can also cause human infections (Karmali and Skirrow 2018). *Campylobacter* is a microaerophilic organism. Isolation of *Campylobacter* from clinical specimens, primarily fecal samples, involves direct plating of the specimen (nonenriched) onto selective media, which prevents the overgrowth of other bacteria, and the use of a microaerobic (5% O2, 10% CO2, 85% N2) environment (Nachamkin and Nguyen 2017). Most clinical laboratories routinely culture specimens for *Campylobacter* using conditions that were developed

and favor isolation of *C. jejuni* and *C. coli*; no single culture method is currently available to isolate all Campylobacter species. Clinical isolation of *Campylobacter* is difficult due to slow growth, and laborious work to identify. Recently, several different methods are available for detection which includes: culture, immunoassays, and molecular tests by PCR (Wang, Clark et al. 2002).

2.4.3. *Clostridium perfringens*

Cl. perfringens is among the most dangerous pathogens that can cause food poisoning. It is an obligate anaerobe that is relatively tolerant of oxygen and can be found in low numbers in the alimentary tract of poultry. It is a spore-forming organism that produces spores that are heat-resistant. Unlike non-spore forming vegetative bacterial species, their spores are not destroyed by cooking and subsequently germinate and overgrow to pathogenic levels if the post-cooking storage is inadequate (Juneja, Baker et al. 2013). When present in meat, growth is favored by conditions in which oxygen has been dispelled by cooking. However, if the meat is kept below 15ºC, the growth of the organism cannot continue and the problem is easily avoided by good refrigeration. Microbiological isolation and characterization of *Cl. perfringens* is difficult due to slow growth. Rapidly detect and quantify methods are available for molecular detection (Wise and Siragusa 2005).

2.4.4. *Escherichia coli (E. coli)*

E. coli is frequently detected in poultry production and processing environments. The toxin-producing strains of *E. coli* can cause diarrhea and hemorrhagic enteritis in humans; this infection can lead to serious consequences such as hemolytic uremic syndrome and thrombotic thrombocytopenia. *E. coli* isolates are mostly part of the normal enteric flora that is present in

animals and often identified in food production, processing, and distribution environments. *E. coli* O 157 is the most important human pathogen, which accounts for almost all major foodborne outbreaks in Europe and the USA (Mead 2004). *E. coli* O 157 can persists in poultry intestine without causing illness in the birds, survives well in soil and is found more often in commercial broiler flocks.

2.4.5. *Listeria monocytogenes*

L. monocytogenes is a Gram-positive facultative anaerobic bacterium. It has the ability to grow at low temperatures even at 0°C. This allows the multiplication at refrigeration temperatures, increasing its ability to evade control in human foodstuffs. (Ramaswamy, Cresence et al. 2007). *L. monocytogenes* are prevalent on raw poultry meat and have been found in chicken, turkey, and pheasant. It has been reported that more than 50% of processed chicken carcasses are likely to be positive (McLauchlin, Mitchell et al. 2004). Listeriosis is a food-related disease causing mortality and morbidity in humans, and the majority of cases are believed to be foodborne. Listeriosis clinical signs vary widely and infcetion is more frequent among immunocompromised people (Kendall, Val Hillers et al. 2006). The importance of the disease arises from cross-contamination in the kitchen with the contaminated raw poultry meat. The organism has the ability to spread to cooked foods or other ready-to-eat items such as salad vegetables and to grow undercooling temperature. Although normal cooking destroys *listerias*, recontamination can occur during post-cooking handling, enhance the potential of the disease. As the pre-cooked items are not necessarily reheated by consumers before being eaten, and the organism is capable of growth under chill conditions, strict microbiological limit values are considered necessary (Ramaswamy, Cresence et al. 2007).

2.4.6. *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive coccus, facultative anaerobic, none spore-forming bacteria belong to the micrococcaceae families which have spherical cells with grape like clusters. *S. aureus* can ferment mannitol to acid and can produce protein A, lipase, coagulase, and hemolysin are produced as well (Bhatia and Zahoor 2007). *S. aureus* can grow under wide range of temperatures from 7 to 48°C, pH ranging from 4.2 to 9.3 and high sodium chloride concentrations of 15%. *S. aureus* is destroyed at pasteurisation treatment but the enterotoxin produced by the bacteria which is heat-resistant is one of the most prevalent causes of clinical food poisoning (Bhatia and Zahoor 2007). Poultry meat can be contaminated during processing by handling by people carrier of *S. aureus* who follow poor hygiene practices, or contaminated equipments (Bortolaia, Espinosa-Gongora et al. 2016). The clinical signs of human *S. aureus* food poisoning includes: nausea, vomiting, diarrhea, salivation, headache, sweating, and fever (De Boer, Zwartkruis-Nahuis et al. 2009). In addition, *S. aureus* cause disease in poultry, such as skin dermatitis, arthritis, foot abscesses (Mead and DODD 1990).

2.4.7. *Proteus*

Proteus is a Gram-negative, aerobic, non-spore forming bacillus. It's of importance to differentiate *Proteus* from *Salmonella* through the urease test (Steinbach and Shetty 2001). *Proteus spp.* have a saprophyte role in decomposing; they are also part of normal flora in the human and poultry intestinal tract. This can be a source of contamination with poultry meat in the slaughterhouse (Nemati 2013). *Proteus* has the ability to utilize the glucose and lactate present in meat aerobically at different temperatures (1 to 25 ^{o}C) to produce nitrogenous compounds responsible for spoilage and off-odors of the meat (Nychas, Skandamis et al. 2008).

In addition, *Proteus* is considered an opportunistic pathogen implicated for septic infections and urinary tract infection (Coker, Poore et al. 2000).

Chapter III Materials and Methods:

3.1 Study area

The current cross-sectional study was carried out during the period from April to June 2019. The study has targeted three cities: Northern Palestine (Tulkarm), Middle of Palestine (Ramallah) and Southern Palestine (Hebron). Thirty samples were collected from each city from both large scale slaughterhouses and small scale slaughterhouses.

3.2 Research Questionnaire:

The research questionnaire was designed to analyze the correlation between the bacterial count and type recovered after different slaughter processes from different cities. The questionnaire was also designed to investigate the legal and technical issues that might be correlated to bacterial count and types of bacteria during slaughtering processes. The questionnaire was revised and audited by: Dr. Azzam Yahia (Slaughterhouse Manager-Palestine Poultry Company "AZIZA"), and Dr. Belal Abu Helal (Instructor at Department of Veterinary Medicine - Faculty of Agriculture and Veterinary Medicine at An Najah National University, Palestinian Veterinary association captain) and Dr. Abdulrahman Ahmad (Ministry of Agriculture).

3.3. Sample Collection

A total of 90 samples were collected throughout this study. The samples were collected from the final product ready to be marketed for human consumption. Each sample consisted of two swabs (COBAN Swab applicator®, Italy) covering the entire inner and outer surface of the processed carcass. The slaughterhouses were given codes *viz*, T, R, and H for Tulkarm, Ramallah and Hebron respectively. The type of processing was also coded as S (for small scale) and L (for

large scale). The collected swabs were transported immediately in the icebox to the laboratory by following standard procedures. The collected swabs from each carcass were transferred to sterile Eppendorf tubes containing 0.5 ml of peptone water and mixed properly by vortex, pooled together in one tube and the mixture of swabs from the same sample was used for bacterial counting, bacterial identification, and molecular characterization.

3.4 Bacteriological Analysis of Poultry

To investigate the type and count of the bacteriological contamination of the poultry slaughter process, the total viable count (TVC) and isolation of *E. coli, Salmonella, Proteus and S. aureus* were detected using different enrichment and selective media. The samples were analyzed within 2-6 hours of collection. The Buffered Peptone Water and the different media (Nutrient Agar (NA), SS Agar (Salmonella-Shigella Agar), Mannitol Salt Agar Base (MSA), and (MacConkey Agar) were prepared according to the manufacturer's instruction.

3.4.1. Preparation of Buffered Peptone Water

Buffered Peptone Water was used to dilute the samples for the bacterial count. Briefly, buffered Peptone Water prepared by suspending 10gm Peptone Water in 500ml distilled water in a beaker and boiled to dissolve completely. The Peptone water was sterilized by autoclaving at 121°C for 20 minutes at 15 psi.

3.4.2. Preparation of bacteriological media

All the reagents for bacterial media preparation were purchased from (Oxoid, Germany and Hi-Media Laboratories, Mumbai). The Nutrient Agar, Mannitol Salt Agar Base (MSA), MacConkey Agar, Salmonella-Shigella Agar (SS Agar) were prepared by suspending 14gm, 55.5gm, 26gm, and 31.5 gm respectively in 500 ml distilled water in a beaker and boiled to dissolve completely.

The media were sterilized by autoclaving at 121 ^{o}C for 20 minutes at 15lbs, except for SS Agar which was not autoclaved or overheated, poured into each Petri dish and kept on the bench for a while with slightly opened lid cover to prevent contamination and to allow for laminar airflow. Chocolate Agar media were kindly provided from the Red Crescent Hospital Laboratory.

3.4.3.Total viable count

Total Viable Count (TVC) is a quantitative estimation for the concentration of microorganisms in the sample. Bacterial counts were represented by colony forming units (CFU) per sample. For evaluating total viable count (TVC), the following technique was followed (Greenwood, Coetzee et al. 1984). A tenfold dilution was prepared by transferring 100 µl of the pooled swabs with 900 µl of sterile peptone water. Dilutions were standardized for further procedures. A quantity of 100 µl inoculums from dilutions 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} were plated on Nutrient agar and spread by 2mm sterile glass beads. Plates were incubated overnight at 37 ºC. The total viable count was calculated by using the standard formula (Greenwood, Coetzee et al. 1984). Plates of the dilution containing 30-300 colonies were counted and TVC was determined by using the proper dilution factor. The Bacterial colonies were counted by the colony counter.

3.5. Bacterial Analysis.

3.5.1 Identification of *Escherichia coli*

A sterile loopful from the pooled samples and streaked on MacConkey agar (Oxoid, Germany). Plates were incubated overnight at 37 ºC. Rapid lactose fermenting colonies of *E. coli* appear dry, circular, moist, smooth, flat, dark pink in color and are surrounded with a darker pink area of precipitated bile salts. Colonies with metallic sheen were considered as positive for *E. coli* (Carter and Cole Jr 2012).

3.5.2. Identification of *Salmonella*

For identification of *Salmonella*, a loopful was taken from the pooled samples and streaked on Salmonella-Shigella Agar (SS Agar) (Hi-Media Laboratories, Mumbai). Plates were incubated overnight at 37 ºC. Non-lactose-fermenting organisms appear as transparent or translucent colorless colonies with black centers that were considered as presumptive positive for *Salmonella*. (Carter and Cole Jr 2012)

3.5.3. Identification of *Proteus*

A loopful from culture was taken from the black colonies that already growth on (SS Agar) and streaked on Chocolate agar (PD-013 CHOCO). Plates were incubated overnight at 37 ºC. Pale or colorless non-lactose fermenting Colonies with swarming motility features were considered positive for *Proteus*, while colonies with no swarming motility features were considered positive for *Salmonella* (Carter and Cole Jr 2012).

3.5.4. Identification of *Staphylococcus aureus*

A loopful culture was taken from pooled samples and streaked on Mannitol salt agar (MSA) (Hi-Media Laboratories, Mumbai). Plates were incubated overnight at 37 ºC. Yellow colonies with yellow zones were considered positive for *S. aureus*. Further confirmation by coagulase on staphylase test was performed. (Carter and Cole Jr 2012)

3.6. Bacterial Storage:

Long term storage of bacteria for further work was done by placing a loopful of the culture in tryptic soy broth containing 10% glycerol and stored at -20 C.

3.7. Molecular Characterization:

Multiplex PCR was performed for the detection and differentiation of *Clostridium perfringens, Listeria monocytogenes,* and *Campylobacter.* A different set of primers were used to specifically amplify certain genes of the target bacteria as mentioned in (Table 3).

Table 3: Primers sequences and amplicon sizes of certain genes for targeted Bacteria.

Bacteria	Gene	Primer Seq	Amplicon size bp	Reference
Listeria monocytogenes	hlyA gene	F: 5'-CGGAGGTTCCGCAAAAGATG-3' R:5'-CCTCCAGAGTGATCGATGTT-3'	234	(Khan, Rathore et al. 2013)
Clostridium perfringens	16S rRNA gene	F:5- AAAGATGGCATCATCATTCAAC-3 R:5- TACCGTCATTATCTTCCCCAA	270	(Wu, Zhang et al. 2009)
Campylobacter	16S rRNA	F :5'-CTGCTTAACACAAGTTGAGTAGG-3 R:5'-TTCCTTAGGTACCGTCAGAA-3'	300	(Uyttendaele, Schukkink et al. 1994)

3.7.1. DNA Extraction:

DNA was extracted from 250 µl of a pooled sample by the boiling method as described previously (Queipo-Ortuño, De Dios Colmenero et al. 2008). In brief, all samples were centrifuged at 15,000 rpm for 15 min, the supernatant was removed and 250 ul of distilled water was added to the pellet and placed in Eppendorf tube, the 250 ul of pooled samples was heated at 97 C for 12 min, followed by 5 min incubation on ice. The Eppendorff tube was centrifugated at 15,000 rpm for 2 min, and 200 ul supernatant was collected and transferred to new Eppendorff tube. All the samples were tested on Nano drop for the concentration and ratio of DNA, results were recorded (Table 18). DNA positive control was also extracted from reference strains of

Listeria monocytogenes, and *Campylobacter* gifted from Istishari Arab Hospital, and *Clostridium perfringens* was extracted from Syva vaccine.

3.7.2. PCR:

5 µl DNA template was mixed with 12.5 µl primers (10 µmol) for each bacterium, dNTP, 10 x buffers, *Taq*, and up to 25 µl final with water. The PCR was performed in (T100 Biorad) according to the following thermocycling condition: initial denaturation of 95 C or 3 min, followed by 34 cycles denaturation at 95 C for 35 sec, annealing at 52 C for 40 sec with increment every cycle 0.2 ºC, and extension of 72 C for 40 sec. The final extension was performed at 72 ºC for 5 min. PCR products were separated on 2% agarose gel by electrophoresis (85 watts) for 1 hour. DNA from references positive control were also run parallel to the samples as a control for DNA isolation and PCR protocol.

3.8. Data collection and Analysis:

Microbial analysis of poultry slaughter processes was done by estimating total viable count (TVC). The total bacterial counts were recorded as CFU/carcass in samples collected from the entire inside and outside of the carcass from small and large scale slaughterhouses from the three cities. *E. coli, Salmonella spp., Proteus and Staphylococcus aureus*. Identification was done by using selective media and relative testing. The presence of characteristic colonies was taken as presumptive positive samples and results were confirmed and recorded accordingly. Percentages of positive samples were calculated and correlated with the data collected in the questionnaire. All data analyzed statistically by using the application of Statistical Package for the Social Sciences (SPSS)(Spss 2011). The following tools of SPSS used:

3.8.1. Cronbach's Alpha:

Cronbach's Alpha of SPSS is an equation for assessing the reliability statistics in use today (Cronbach 1951). It determines the internal consistency or average correlation of items in a survey instrument to gauge its reliability.

$$\alpha = \frac{N\bar{c}}{\bar{v} + (N-1)\bar{c}}$$

Here α is the Alpha coefficient, N is equal to the number of items, $\bar{c}$ is the average inter-item covariance among the items and $\bar{v}$ equals the average variance.

The alpha coefficient ranges in value from 0 to 1 and used to describe the reliability of factors extracted from formatted questionnaires or scales with dichotomous and multi-points. The higher the score, the more reliable the generated scale is. Ratio with 0.7 or higher has been indicated to be an acceptable reliability coefficient and could be used for further analysis.

Table 4: Interpreting alpha for dichotomous questions (i.e. questions with two possible answers). Adapted from (Cronbach 1951):

Cronbach's alpha	Internal consistency
$\alpha \geq 0.9$	Excellent
$0.9 > \alpha \geq 0.8$	Good
$0.8 > \alpha \geq 0.7$	Acceptable
$0.7 > \alpha \geq 0.6$	Questionable
$0.6 > \alpha \geq 0.5$	Poor
$0.5 > \alpha$	Unacceptable

3.8.2. Correlation test:

Correlation test is a statistical analysis that measures the association between two variables by determining the strength and the direction of the relationship (Baird 2014). The value of the correlation coefficient varies between +1 and -1. The value of 1 indicates a perfect degree of association between the two variables while decreasing of correlation coefficient value towards 0, the relationship between the two variables will be weaker. Sign of the coefficient either positive or negative indicates the direction of the relationship, a + sign indicates a positive relationship while a – sign indicates a negative relationship between the two variables.

The correlation test of SPSS was used in this study to measures the relationship between the legal regulation practice, technical issues and the presence and degree of bacterial contamination in the dressed poultry carcass during the different slaughtering processes.

3.8.3. ANOVA test:

Analysis of variance (ANOVA) is a statistical technique that is used in order to make a reliable and confident decision (Girden 1992). ANOVA of SPSS was used to determine if TVC experiment results are significant or not by comparing the means different slaughters scale method and city groups are different significantly from each other or not.

3.8.4. Mann-Whitney Test:

The Mann-Whitney U test is a statistical tool of SPSS that could be used to compare differences and determine their presence between two independent groups when the dependent variable is either ordinal or continuous (Ruxton 2006). The percentage and type of bacteria between the different slaughters scale method and city groups were analyzed to determine if they are different significantly from each other.

List of materials:

Table 5: List of materials, Manufacturer Company, and catalog number

Name of material	Manufacturer company	Catalog number
Disposable Swab	Copan	H219DD
Buffered Peptone Water	OXOID	CM0509
MacConkey Agar	OXOID	CM0007
Salmonella-Shigella Agar (SS Agar)	HIMEDIA	M108
Nutrient Agar	OXOID	CM0003
Mannitol Salt Agar (MSA)	HIMEDIA	M118
Chocolate Agar		10227302
Glass Beads 2 mm	MERCK	104017
Thermocycler	Biorad	T100

Chapter IV: Results

4.1. Descriptive Analysis

The present study was conducted to compare the bacterial contamintion; type and count between final products of large scale and small scale slaughtering processes in Palestine. A total of 90 swabs samples were taken from final dressed poultry carcasses from 10 facilities (5 large scale slaughterhouses and 5 small scale slaughter stores). The samples were collected from three main cities according to the following table:

Table 6: Classification of facilities and sample numbers according to targeted Cities and type of slaughtering scale.

	Large scale slaughterhouses		Small scale slaughter stores		Total
	No. of facilities	No. of samples	No. of facilities	No. of samples	
Tulkarm	2	15	2	15	30\4
Ramallah	2	20	1	10	30\3
Hebron	1	15	2	15	30\3
Total	5	50	5	40	90\10

4.2. Microbial analysis of poultry slaughter carcasses

4.2.1. Total viable count

Total viable counts (TVC) were estimated from all samples (N=90). A quantity of 100 µl inoculums from dilutions 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} were plated on Nutrient agar and counted after overnight incubation at 37 ºC (Fig 3).

Figure 3: Nutrient agar plate incubated overnight at 37 ºC for counting

It was observed that the TVC values ranged from $1.6*10^{1}$CFU/carcass to $2.2*10^{7}$ CFU/ carcass in all tested samples.

For small scale slaughterhouses; the minimum TVC was $1.6*10^{1}$CFU/carcass and the maximum was $2.2*10^{7}$ CFU/ carcass. In large scale slaughterhouses; the minimum TVC was $8.3*10^{1}$CFU/carcass and the maximum was $2.04*10^{7}$ CFU/ carcass (Table 7).

Table 7: Descriptive analysis of TVC in small and large scale slaughterhouses

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Small Scale	40	770629.25	3499568.165	553330.311	160	22100000
Large Scale	50	567685.00	2871417.734	406079.790	830	20400000
Total	90	657882.44	3149022.176	331936.083	160	22100000

The mean TVC value of the small scale slaughterhouses was $7.7*10^5$ and for the large scale $5.6*10^5$. However, this difference was not significant at $P > 0.05$ ($P = 0.763$). The results were shown in (Table 8).

Table 8: Differences between TVC means of small and large scale slaughterhouses.

TVC, ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	915252635734.72	1	915252635734.722	.091	.763
Within Groups	881639066692927.5	88	10018625757874.176		
Total	882554319328662.2	89			

The estimated TVC in both small and large scale slaughterhouses was varied between the studied cities, the lowest mean bacterial contamination in the two facilities type was observed in Tulkarm with a TVC mean of $1*10^4$ and the highest was recorded in Ramallah with a mean TVC of $1.7*10^6$ (Table 9).

Table 9: Descriptive analysis of TVC in targeted cities

TVC, Descriptive

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Tulkarm	30	10354.00	17817.749	3253.061	3700.74	17007.26	160	86000
Hebron	30	236146.67	106850.833	19508.204	196247.91	276045.42	29400	450000
Ramallah	30	1727146.67	5349829.558	976740.776	-270512.52	3724805.85	3900	22100000
Total	90	657882.44	3149022.176	331936.083	-1667.48	1317432.37	160	22100000

There is only one significant difference in which the TVC estimated in Tulkarm was significantly lower that TVC estimated in Ramallah ($P < 0.05$).

Dependent Variable: TVC, LSD

(I) Adress_City	(J) Adress_City	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Tulkarm	Hebron	-225792.667	797668.977	.778	-1811246.13	1359660.80
	Ramallah	-1716792.667*	797668.977	.034	-3302246.13	-131339.20
Hebron	Tulkarm	225792.667	797668.977	.778	-1359660.80	1811246.13
	Ramallah	-1491000.000	797668.977	.065	-3076453.47	94453.47
Ramallah	Tulkarm	1716792.667*	797668.977	.034	131339.20	3302246.13
	Hebron	1491000.000	797668.977	.065	-94453.47	3076453.47

*. The mean difference is significant at the 0.05 level.

4.2.2. Detection of *E. coli*

E. coli are bacteria that are present in the digestive tracts of poultry (Yeoman, Chia et al. 2012). In the present study, all poultry slaughter carcasses samples collected from small scale slaughter stores (N=40) showed the presence of colonies of characteristic dry, circular, moist, smooth, flat, pink color, the metallic sheen on MacConkey agar indicating the presence of *E. coli* (Fig 4).

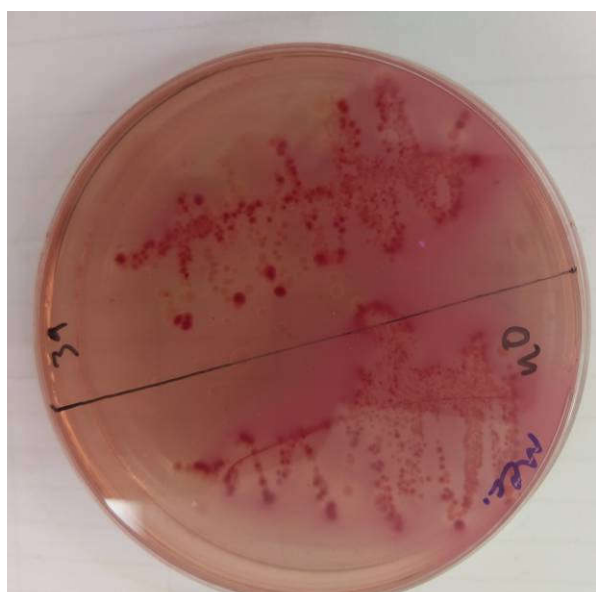

Figure 4:Identification of *E. coli* colonies of characteristic metallic sheen on MacConkey agar

In contrast, 47 samples out of 50 collected from large scale slaughterhouses were positive. No significant differences in the percentage of positive *E. coli* were observed between the two types of facilities (P>0.05).

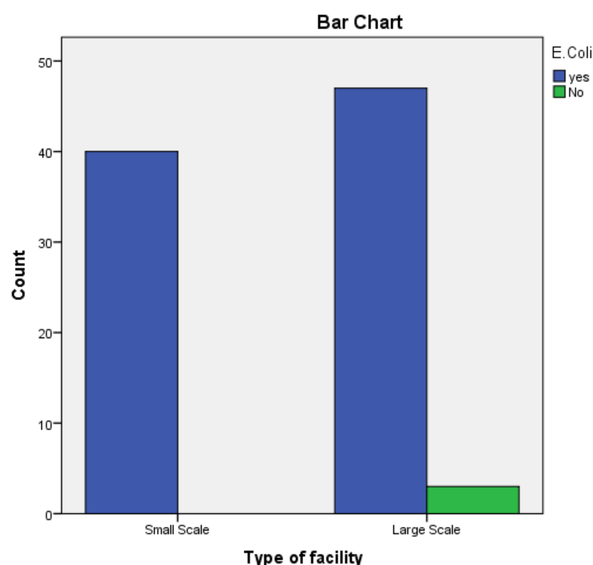

Figure 5: Detected *E. coli* Bacteria in small and large scale slaughterhouses

4.2.3. Detection of *Salmonella spp*.

Salmonella is an important organism of public health significance (Humphrey 2000). All the samples were selectively plated on SS Agar for screening against *Salmonella*. Samples with non-lactose-fermenting organisms appear as transparent or translucent colorless colonies with black centers that were considered as presumptive positive for *Salmonella* (Fig 6).

Figure 6: Identification of *Salmonella* characterized with black centers colonies on SS agar

47.5% of the samples from small scale slaughter stores were positive compared to 46% of the large scale slaughterhouses. No significant differences in the percentage of positive Salmonella were observed between the two types of facilities ($P>0.05$) (Table 10).

Table 10: Detected *Salmonella* in small and large scale slaughterhouses

Type of facility * *Salmonella* Crosstabulation

		Salmonella		Total
		Yes(**%**)	No(%)	
Type of facility	Small Scale	19 (**47.5%**)	21(52.5%)	40
	Large Scale	23(**46 %**)	27 (**54%**)	50
Total		42	48	90

4.2.4. Detection of *Proteus*

Proteus organisms are implicated as serious causes of infections in humans, It has been reported as one of the causative agents of human pneumonia and other lung infection conditions (Guentzel 1996). All samples that shown presumptive positive *Salmonella* were selectively plated on Chocolate Agar for differentiation between *Salmonella* and *Proteus*, Pale or colorless

non-lactose fermenting Colonies with swarming motility features were considered positive for *Proteus*, while colonies with no swarming motility features were considered positive for *Salmonella* (Fig 7).

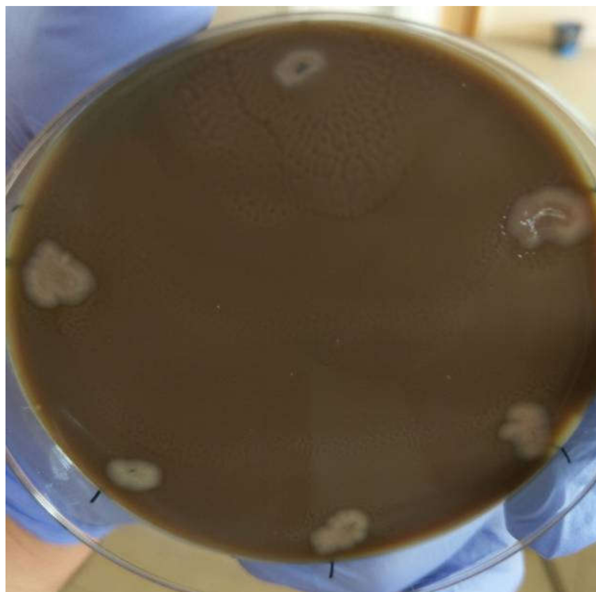

Figure 7: Identification of *Proteus* characterized with swarming features colonies on Chocolate agar

15 % of the samples from small scale slaughter stores were positive compared to 12 % of the large scale slaughterhouses. No significant differences in the percentage of positive *Proteus* were observed between the two types of facilities (P>0.05) (Table 11)

Table 11: Detected *Proteus* in small and large scale slaughterhouses

Type of facility * *Proteus* Crosstabulation

		Proteus		Total
		Yes	No	
Type of facility	Small Scale	6 (**15 %**)	34 (**85 %**)	40
	Large Scale	6 (**12 %**)	44 (**88 %**)	50
Total		12	78	90

4.2.5. Detection of *S. aureus*

Staphylococcus is part of the normal skin and mucosal flora (Davis 1996), many infections are the result of a wound, mucosal damage, or both (Scales and Huffnagle 2013). All the samples were selectively plated on MSA agar for screening against *S. aureus*. Samples with yellow colonies and yellow zones were considered positive for *S. aureus*. (Fig 8).

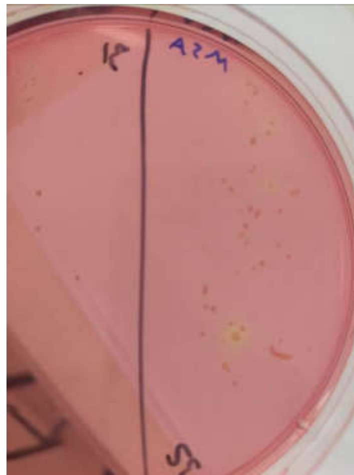

Figure 8: Identification of *S. aureus* of characteristic yellow colonies on MSA agar

Further confirmation by coagulase on staphylase test was performed. 42.5 % of the samples from small scale slaughter stores were positive compared to 58 % of the large scale slaughterhouses. No significant differences in the percentage of positive *S. aureus* were observed between the two types of facilities (P>0.05) (Fig 9).

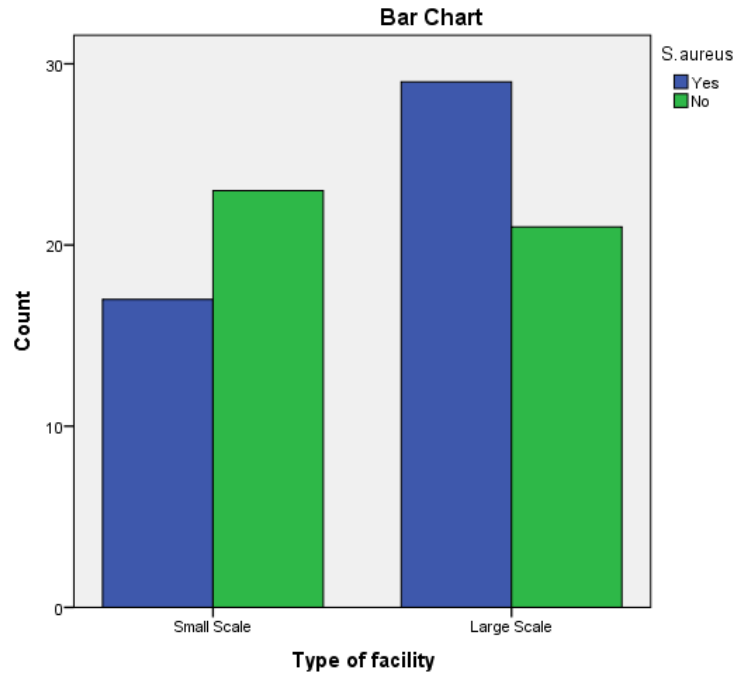

Figure 9: Detected *S. aureus* in small and large scale slaughterhouses

4.3. Molecular detection of *Campylobacter*, *Clostridium*, and *Listeria monocytogenes*

Multiplex PCR was performed for the differential detection of *Clostridium perfringens, Listeria monocytogenes,* and *Campylobacter spp.*. Specific primers were used as described earlier (Fig 10).

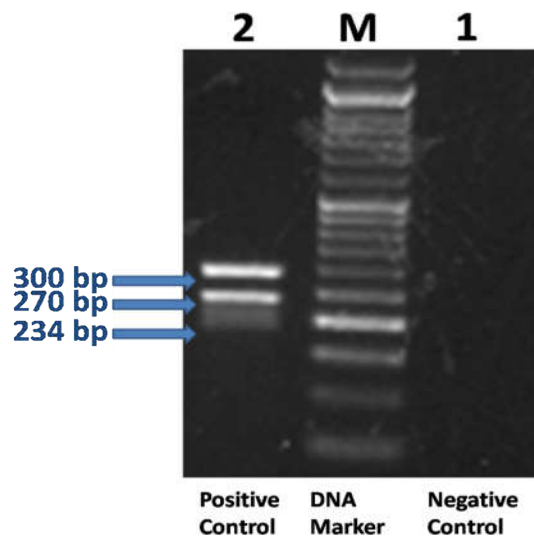

Figure 10: Molecular detection of primer positive controls.

The results showed that all the tested samples were negative for *C. perfringes* (amplicon size 270 bp) and *L. monocytogens* (amplicon size 234 bp) (data is not shown). However, our results showed significant differences in the presence of *Campylobacter* between a small scale (20%) compared to the large scale (6%) ($P < 0.05$). (Figure 11).

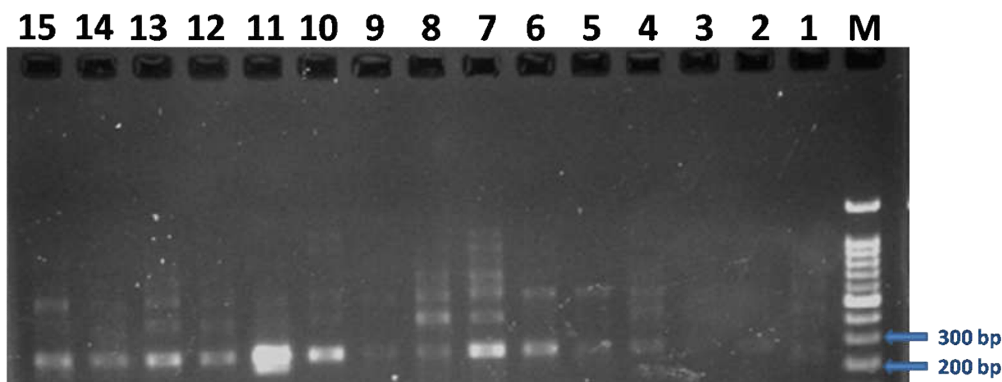

Figure 11: Molecular detection of Campylobacter in tested sampls. M: DNA marker, Lanes1-15: tested samples

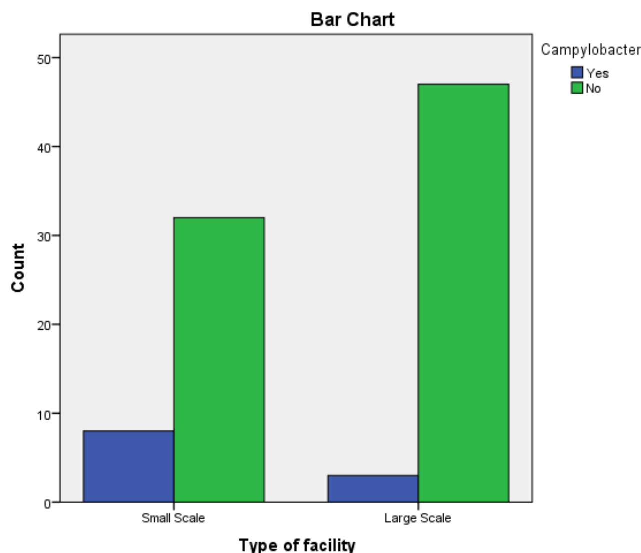

Figure 12: Detection of Campylobacter in small and large scale slaughterhouses.

4.4. The Correlation between type of facility and bacterial contamination of poultry carcass

A questionnaire was designed for this study and data was collected to investigate the differences of the facility type reflecting the poultry carcass possessing and bacterial contamination of the final product. Initially, the questionnaire was tested with Cronbach's Alpha estimations (reliability test). Cronbach's Alpha was 0.899 which is good and data obtained of this study is reliable and could be used for further analysis of the findings.

Table 12: Cronbach's Alpha estimations.

Reliability Statistics

Cronbach's Alpha	N of Items
.899	66

4.5. Effect of Facility space, Number of working personnel, Daily working hours and production capacity on the bacterial contamination:

No correlation between the facility space and the quantity and type of bacterial contamination were detected in this study. In contrast, increase daily working hours resulted in an increase the presence of *S.aureus* and *Salmonella* in the final poultry products. However, increase production capacity decreases the presence of *S.aureus*, *Salmonella, and Campylobacter* in the final poultry products. Besides, we found that numbers of working personnel were positively associated with the presence of *E. coli* and *Campylobacter* in the final products (Table 13)

4.6. Effect of legal and registration issues on Bacterial contamination:

The registration of the facility in the governmental authorities showed a negative correlation value in which the presence of *Salmonella* and *Campylobacter* decreased significantly. The presence of supervisor veterinarian for the whole process which also made an inspection of live chickens before slaughtering, holding a Palestinian Standards Institution (PSI) certificate as well as testing of all employees in a health center before working in the facility has decreased the presence of *E. coli*, *Salmonella,* and campylobacter. Holding a certificate from 3rd party body and presence of facility own laboratory decreased the presence of *S.aureus* and *Salmonella*. in addition, inspection and certification of chickens by the governmental authority before slaughtering decreased the presence of *Campylobacter*. The growing of the live chickens in the company's own farm decreased the presence of *E. coli*. We found that there were no records at the small and large scale for the medication given to the live chicken's (Table 14).

4.7. Effect of the facility infrastructure and departments on Bacterial contamination:

The connection of the facility with the municipality water supply resulted in a negative correlation value, i.e. the presence of *Salmonella* decreased significantly. The presence of a standby electricity generator, separate area for slaughtering process, separate area for storage of final products and the maintenance of slaughtering floor to prevent stagnant water and dirt to be stuck has decreased the presence of *E. coli*, *Salmonella*, and *Campylobacter*. The connection of facility with municipality sewage system decreased the presence of *S.aureus*, *Salmonella*, and *campylobacter*. The presence of a separate area for the reception of live chickens, a cooling system containing an alarm in case of temperature fluctuation, a separate area to deal with the customers and a pest control system in the facility decreased the presence of *Campylobacter*. Also, the need for permission for entering to the slaughtering area decreased the presence of *E. coli*. The air filtration system in the facility decreased the presence of *E. coli* and *Salmonella*. A contract with a 3rd party body for pest control services in the facility decreased the presence of *S.aureus* and *Salmonella*. the presence of a cooling system in the area for the storage of final products has shown no correlation with the presence of any type of bacteria. (Table 15).

4.8. Effect of slaughtering process on the Bacterial contamination:

Interestingly, the fasting of live chickens before slaughtering has shown a positive correlation with the presence of S.*aureus*, *Salmonella,* and *Campylobacter*, a significant increased in their presence was noted. Stunning of live chickens before slaughtering and also the testing of concentration for used chemical materials during the chilling process decreased the presence of *S.aureus* and *Salmonella* significantly. Changing of scalding water after every batch and inspection of chicken after evisceration significantly decrease the presence of *E. coli* and

Campylobacter, while increased the presence of *S.aureus*. Noticing any marks of viscera laceration in the evisceration process decreased the presence of *Salmonella* and *Campylobacter*. Water chilling and washing the carcass decreased the presence of *Campylobacter*. Using chemical materials during washing before chilling and also taking samples during processing for laboratory tests has decreased the presence of *E. coli* and *Salmonella*. Using chemical materials during the water chilling process and measuring the temperature after the chilling process has decreased the presence of *E. coli*, *Salmonella*, and *Campylobacter*. Detection of any marks for feathers or non-edible parts after the chilling process has shown an increase in the presence of *E. coli* and decrease in the presence of *Campylobacter*. All the samples were collected from fully bled chickens after slaughtering. (Table 16).

4.9. Effect of Manufacturing practice and cleaning on Bacterial contamination:

The commitment of all employees in a good manufacturing practice (GMP) and the usage of antiseptic in the cleaning process decreased the presence of *salmonella* and *campylobacter* significantly. Training of employees about quality and GMP decreased the presence of *S.aureus* and *Salmonella*. Cleaning after every batch and having separate teams before and after the chilling process decreased the presence of *E. coli* and *Campylobacter*, while increased the presence of *S.aureus*. Following the employees for a clear cleaning process even with a chance of using tools and equipment in different areas or departments has decreased the presence of *E. coli*. Using detergents in the cleaning process has decreased the presence of *E. coli*, *Salmonella*, and *Campylobacter*. Even with a chance of different teams to be mixed in different departments, there is a decrease in presence of *Campylobacter*, while differentiation between these teams to avoid mixing between them has shown a decrease in the presence of *E. coli* and *Salmonella*.

Taking a swab test for the facility floor and surfaces has decreased the presence of *E. coli* with an increase in the presence of *S.aureus*. In addition, There was an increase in the presence of *S.aureus* despite the cleaning of the equipment after every production batch. The correlation of using hot water in the cleaning process and spending more than 15 minutes in the cleaning could not be computed because at least one of the variables is constant. (Table 17).

Table 13: Effect of Facility space, Number of working personnel, Daily working hours and production capacity on the bacterial contamination.

		TVC	*E. coli*	*S.aureus*	*Salmonella*	*Proteus*	*Campylobacter*	*C.perfringens*	*L.monocytogenes*
Facility_space	Pearson Correlation	.075	.207	-.143	.035	.141	.024	.[b]	.[b]
	Sig. (2-tailed)	.485	.050	.180	.743	.184	.819		
Number_of_working_personnel	Pearson Correlation	-.081	.260*	.009	.187	.118	.251*	.[b]	.[b]
	Sig. (2-tailed)	.446	.013	.934	.077	.269	.017		
Working_hours	Pearson Correlation	-.060	-.054	.297**	.272**	.114	.108	.[b]	.[b]
	Sig. (2-tailed)	.574	.614	.004	.010	.285	.309		
Daily_production_capacity	Pearson Correlation	.150	.152	-.307**	-.259*	.104	-.306**	.[b]	.[b]
	Sig. (2-tailed)	.157	.153	.003	.014	.330	.003		

Table 14: Effect of legal and registration issues on Bacterial contamination.

		TVC	*E. coli*	*S.aureus*	*Salmonella*	*Proteus*	*Campylobacter*	*C.perfringens*	*L.monocytogenes*
Is the facility has been registered in the governmental authority?	Pearson Correlation	.127	-.115	-.160	-.464**	.024	-.602**	.[b]	.[b]
	Sig. (2-tailed)	.232	.280	.132	.000	.820	.000		
Does the facility contain a veterinarian doctor to supervision the whole process?	Pearson Correlation	-.006	-.233*	.051	-.244*	-.112	-.298**	.[b]	.[b]
	Sig. (2-tailed)	.952	.027	.635	.021	.294	.004		
Does the facility hold a PSI certificate?	Pearson Correlation	-.006	-.233*	.051	-.244*	-.112	-.298**	.[b]	.[b]
	Sig. (2-tailed)	.952	.027	.635	.021	.294	.004		
Does the facility hold a quality certificate from 3rd party body?	Pearson Correlation	.060	.054	-.297**	-.272**	-.114	-.108	.[b]	.[b]
	Sig. (2-tailed)	.574	.614	.004	.010	.285	.309		
Does the facility contain its own laboratory to do the tests for raw material and finish goods?	Pearson Correlation	.060	.054	-.297**	-.272**	-.114	-.108	.[b]	.[b]
	Sig. (2-tailed)	.574	.614	.004	.010	.285	.309		
Are the live chickens being inspected and certified by any governmental authority before slaughtering?	Pearson Correlation	.153	-.090	.153	-.061	.189	-.513**	.[b]	.[b]
	Sig. (2-tailed)	.149	.401	.151	.570	.074	.000		
Does the veterinarian doctor make an inspection for live chickens before slaughtering?	Pearson Correlation	-.006	-.233*	.051	-.244*	-.112	-.298**	.[b]	.[b]
	Sig. (2-tailed)	.952	.027	.635	.021	.294	.004		
Had the live chickens been grown in the company's own farms?	Pearson Correlation	.143	-.208*	-.065	-.075	-.088	-.129	.[b]	.[b]
	Sig. (2-tailed)	.180	.050	.545	.484	.411	.226		
Is there any record for the medications which were	Pearson Correlation	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]

given to the live chickens?	Sig. (2-tailed)								
Were all employees tested in the health center before working in the facility?	Pearson Correlation	.116	-.299**	-.187	-.331**	-.170	-.231*	.[b]	.[b]
	Sig. (2-tailed)	.278	.004	.077	.001	.109	.028		

Table 15: Effect of the facility infrastructure and departments on Bacterial contamination.

		TVC	*E. coli*	*S.aureus*	*Salmonella*	*Proteus*	*Campylobacter*	*C.perfringens*	*L.monocytogenes*
Does the facility connect with the municipality water supply?	Pearson Correlation	-.051	-.083	-.139	$-.299^{**}$	-.088	-.106	.[b]	.[b]
	Sig. (2-tailed)	.631	.436	.191	.004	.411	.319		
Does the facility contain a standby electricity generator?	Pearson Correlation	-.006	$-.233^{*}$	.051	$-.244^{*}$	-.112	$-.298^{**}$	.[b]	.[b]
	Sig. (2-tailed)	.952	.027	.635	.021	.294	.004		
Does the facility connect with the municipality sewage system?	Pearson Correlation	.189	.186	$-.533^{**}$	$-.445^{**}$	0.000	$-.373^{**}$	.[b]	.[b]
	Sig. (2-tailed)	.074	.080	.000	.000	1.000	.000		
Does the facility contain a separate area for the reception of live chickens?	Pearson Correlation	.032	-.166	.154	-.015	-.044	$-.212^{*}$	.[b]	.[b]
	Sig. (2-tailed)	.763	.118	.147	.889	.682	.044		
Does the facility contain a separate area for the slaughtering process?	Pearson Correlation	-.006	$-.233^{*}$	.051	$-.244^{*}$	-.112	$-.298^{**}$	.[b]	.[b]
	Sig. (2-tailed)	.952	.027	.635	.021	.294	.004		
Does the floor of the slaughtering area good maintain to prevent stagnant water and dirt to be stuck?	Pearson Correlation	-.006	$-.233^{*}$	.051	$-.244^{*}$	-.112	$-.298^{**}$	.[b]	.[b]
	Sig. (2-tailed)	.952	.027	.635	.021	.294	.004		
Does the facility contain a separate area for the storage of final products??	Pearson Correlation	-.006	$-.233^{*}$	.051	$-.244^{*}$	-.112	$-.298^{**}$	.[b]	.[b]
	Sig. (2-tailed)	.952	.027	.635	.021	.294	.004		
Does the area for the storage of final products have a cooling system?	Pearson Correlation	-.158	-.186	.089	-.045	-.196	.170	.[b]	.[b]
	Sig. (2-tailed)	.136	.080	.405	.677	.064	.110		
Does the cooling system contain an alarm in case of temperature fluctuation?	Pearson Correlation	.032	-.166	.154	-.015	-.044	$-.212^{*}$	.[b]	.[b]
	Sig. (2-tailed)	.763	.118	.147	.889	.682	.044		

Does the facility contain a separate area to deal with the customers?	Pearson Correlation	.032	-.166	.154	-.015	-.044	-.212*	.[b]	.[b]
	Sig. (2-tailed)	.763	.118	.147	.889	.682	.044		
Does the slaughtering area need the authorization to enter in?	Pearson Correlation	.109	-.250*	.123	.050	-.018	-.065	.[b]	.[b]
	Sig. (2-tailed)	.304	.017	.249	.642	.865	.545		
Does the facility contain an air filtration system?	Pearson Correlation	.085	-.385**	.018	-.224*	-.106	-.180	.[b]	.[b]
	Sig. (2-tailed)	.425	.000	.869	.034	.321	.089		
Does the facility contain a pest control system?	Pearson Correlation	.032	-.166	.154	-.015	-.044	-.212*	.[b]	.[b]
	Sig. (2-tailed)	.763	.118	.147	.889	.682	.044		
Does the facility make a contract with 3rd party body for pest control service?	Pearson Correlation	.060	.054	-.297**	-.272**	-.114	-.108	.[b]	.[b]
	Sig. (2-tailed)	.574	.614	.004	.010	.285	.309		

Table 16: Effect of slaughtering process on the Bacterial contamination.

		TVC	*E. coli*	*S.aureus*	*Salmonella*	*Proteus*	*Campylobacter*	*C.perfringens*	*L.monocytogenes*
Are the live chickens being fasted before slaughtering?	Pearson Correlation	-.155	-.155	.357**	.329**	-.071	.312**	.[b]	.[b]
	Sig. (2-tailed)	.145	.144	.001	.002	.507	.003		
Are the live chickens being stunned before slaughter?	Pearson Correlation	.093	.083	-.457**	-.359**	-.175	-.167	.[b]	.[b]
	Sig. (2-tailed)	.385	.436	.000	.001	.098	.116		
Are the slaughtered chickens being fully bleeding?	Pearson Correlation	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]
	Sig. (2-tailed)								
Do you change the scalding water after every batch?	Pearson Correlation	-.041	-.276**	.225*	-.099	-.052	-.251*	.[b]	.[b]
	Sig. (2-tailed)	.698	.008	.033	.351	.628	.017		
Do you inspect the chicken after evisceration?	Pearson Correlation	-.047	-.284**	.252*	-.126	-.043	-.244*	.[b]	.[b]
	Sig. (2-tailed)	.659	.007	.016	.235	.689	.020		
Do you see any marks for viscera laceration in the evisceration process?	Pearson Correlation	.080	-.141	.040	-.305**	.095	-.490**	.[b]	.[b]
	Sig. (2-tailed)	.454	.184	.708	.003	.373	.000		
Do you wash the carcass before the chilling process?	Pearson Correlation	.032	-.166	.154	-.015	-.044	-.212*	.[b]	.[b]
	Sig. (2-tailed)	.763	.118	.147	.889	.682	.044		
Do you use any chemical material during the washing?	Pearson Correlation	.085	-.385**	.018	-.224*	-.106	-.180	.[b]	.[b]
	Sig. (2-tailed)	.425	.000	.869	.034	.321	.089		
Do you do the water chilling process?	Pearson Correlation	.032	-.166	.154	-.015	-.044	-.212*	.[b]	.[b]
	Sig. (2-tailed)	.763	.118	.147	.889	.682	.044		
Do you use any chemical material during the water	Pearson Correlation	.116	-.299**	-.187	-.331**	-.170	-.231*	.[b]	.[b]

chilling process?	Sig. (2-tailed)	.278	.004	.077	.001	.109	.028		
In the case of using chemical material, do you test the concentration of this chemical?	Pearson Correlation	.060	.054	-.297**	-.272**	-.114	-.108	.[b]	.[b]
	Sig. (2-tailed)	.574	.614	.004	.010	.285	.309		
Do you measure the temperature after the chilling process?	Pearson Correlation	.116	-.299**	-.187	-.331**	-.170	-.231*	.[b]	.[b]
	Sig. (2-tailed)	.278	.004	.077	.001	.109	.028		
Do you see any marks for feathers or non-edible parts after the chilling process?	Pearson Correlation	.087	.276**	-.033	-.045	.193	-.335**	.[b]	.[b]
	Sig. (2-tailed)	.413	.008	.757	.674	.068	.001		
Do you take samples during the process for laboratory tests?	Pearson Correlation	.085	-.385**	.018	-.224*	-.106	-.180	.[b]	.[b]
	Sig. (2-tailed)	.425	.000	.869	.034	.321	.089		

Table 17: Effect of Manufacturing practice and cleaning on Bacterial contamination.

		TVC	*E. coli*	*S.aureus*	*Salmonella*	*Proteus*	*Campylobacter*	*C.perfringens*	*L.monocytogenes*
Do all employees commit to good manufacturing practice?	Pearson Correlation	-.045	.115	-.138	-.232*	-.097	-.231*	.[b]	.[b]
	Sig. (2-tailed)	.671	.280	.195	.028	.362	.028		
Are all employees being trained and aware of quality and GMP?	Pearson Correlation	.065	.058	-.319**	-.214*	-.123	-.117	.[b]	.[b]
	Sig. (2-tailed)	.543	.587	.002	.043	.250	.274		
Do you do the cleaning after every batch?	Pearson Correlation	-.041	-.276**	.225*	-.099	-.052	-.251*	.[b]	.[b]
	Sig. (2-tailed)	.698	.008	.033	.351	.628	.017		
Do you have a clear cleaning process and all employees follow it?	Pearson Correlation	.143	-.208*	-.065	-.075	-.088	-.129	.[b]	.[b]
	Sig. (2-tailed)	.180	.050	.545	.484	.411	.226		
Do you use any detergent in the cleaning process?	Pearson Correlation	-.006	-.233*	.051	-.244*	-.112	-.298**	.[b]	.[b]
	Sig. (2-tailed)	.952	.027	.635	.021	.294	.004		
Do you use any antiseptic in the cleaning process?	Pearson Correlation	-.045	.115	-.138	-.232*	-.097	-.231*	.[b]	.[b]
	Sig. (2-tailed)	.671	.280	.195	.028	.362	.028		
Do you use hot water in the cleaning process?	Pearson Correlation	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]
	Sig. (2-tailed)								
Do you spend more than 15 minutes in the cleaning?	Pearson Correlation	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]	.[b]
	Sig. (2-tailed)								
Do you have separate teams before and after the chilling process?	Pearson Correlation	-.047	-.284**	.252*	-.126	-.043	-.244*	.[b]	.[b]
	Sig. (2-tailed)	.659	.007	.016	.235	.689	.020		
Is there any chance for these	Pearson	.032	-.166	.154	-.015	-.044	-.212*	.[b]	.[b]

teams to be mixed?	Correlation								
	Sig. (2-tailed)	.763	.118	.147	.889	.682	.044		
Do you differentiate between these teams by special dress or marks?	Pearson Correlation	.085	-.385**	.018	-.224*	-.106	-.180	.[b]	.[b]
	Sig. (2-tailed)	.425	.000	.869	.034	.321	.089		
Do you take a swab test for the facility? Floor , roof..etc?	Pearson Correlation	.055	-.525**	.275**	-.047	-.035	-.132	.[b]	.[b]
	Sig. (2-tailed)	.608	.000	.009	.658	.746	.215		
Do you clean the tools and equipments after every production batch?	Pearson Correlation	.000	-.194	.312**	.131	.017	-.153	.[b]	.[b]
	Sig. (2-tailed)	.999	.067	.003	.219	.870	.149		
Is there any chance for these tools or equipments to be used in different areas or departments?	Pearson Correlation	.114	-.244*	.098	.074	-.027	-.073	.[b]	.[b]
	Sig. (2-tailed)	.285	.020	.356	.489	.800	.496		

Chapter V: Discussion

Bacterial content is an important indicator of the quality of poultry meat; our results showed a mean TVC value of the small scale slaughter stores was $7.7*10^5$/ ml of the total area of the inner and the outside of the carcass and $5.6*10^5$/ ml for the large scale slaughterhouses. The TVC was lower in the large scale slaughterhouses, but the difference was not significant at $P > 0.05$ ($P= 0.763$). The detected TVC was varied between the studied cities, in Tulkarm TVC ($1*10^4$) was significantly lower than TVC estimated in Ramallah ($1.7*10^6$) ($P< 0.05$). This might be related to the low TVC detected in the large scale slaughterhouses that implicated high modernized poultry processing compared to the other cities. The Palestinian standards for fresh chilled chicken required less than $1*10^6$ cell/gram as microbial requirements for the validity of fresh chilled chicken. The TVC unit of this study was defined as CFU/carcass surface. This measure reflects the bacterial contamination in the poultry carcass rather than the entire meat, and consider as a potential measurement of the processing procedure (Capita, Prieto et al. 2004).

The TVC means of small scale and large scale was within the maximum limit, $7.7*10^5$ and $5.6*10^5$ respectively (if we considered that the amount of bacterial contamination on the carcass surface represents the amount of contamination in 1 gram of meat). On the other hand, the mean TVC of Ramallah city for both scales was $1.7*10^6$. There were 6 samples (n=90) that have a TVC above the maximum limit. Their TVC ranged from $1.18*10^6$ up to $22*10^6$. (6.6% of the total samples were above the maximum limits) (PSI 2013). In agreement to our findings Cohen et al., 2007 found that the traditional slaughtering process has higher bacterial counts compared to large scale slaughterhouses and the samples from the traditional slaughtering process contained 25% TVC above their maximum limits (Cohen, Ennaji et al. 2007). In another study

in India, it has been found that the highest bacterial counts in poultry meat samples were recorded with the traditional slaughtering process. Also, the prevalence of *Salmonella* in traditional meat shops was higher in the range of 25-65% due to high levels of microbial contamination and the poor hygienic quality of poultry meat processing (Ruban and Fairoze 2011).

In the present study, 100% of samples collected from small scale slaughterhouses (N=40) showed the presence of *E. coli*, while 94% of the sample collected from large scale slaughterhouses were positive. There is no significant difference between the two types of facilities (P>0.05). *E. coli* is widely prevalence in processed poultry meat. Our findings are similar to another study in Iran, where 100% of marketed poultry meat contaminated with *E. coli* (Javadi and Safarmashaei 2011), and even in two large scale slaughterhouses in Sweden (Ternstom and Molin 1987). This can be explained with the wide distribution of *E. coli* in the poultry body (Allen, Corry et al. 2000)

This study has shown that 47.5% of the samples from a small scale were positive for the presence of *Salmonella* compared to 46% of the large scale. No significant differences between the two types of facilities (P>0.05). The Palestinian standards for fresh chilled chicken require a completely devoid of *Salmonella* in 25 grams of meat sample as a microbial requirement for the validity of fresh chilled chicken (PSI 2013). This was not achieved in both scales according to our study results. In a study in Brazil applied on 60 Brazilian small scale poultry slaughterhouses; 42% of carcasses, 23.1% of utensils, 71.4% of water, and 71.4% of freezers and refrigerators samples were positive for *Salmonella*. Furthermore, one of these slaughterhouses was selected to monitor the dissemination of *Salmonella* along the slaughtering process. The result was that all samples collected along the slaughtering process in the selected slaughterhouse

were *Salmonella* positive (Fuzihara, Fernandes et al. 2000). In contrast, all tested samples of marketed poultry meat in Iran have shown no presence of *Salmonella spp* (Javadi and Safarmashaei 2011). Another study has shown that *Salmonella* was not detected in any carcasses between 163 samples New Zealand broiler carcasses (Chrystal, Hargraves et al. 2008). In a study in spain, Carramiñana et al., 1997 has shown that the incidence rate of *Salmonella* for a total of 192 samples diversely ranged between several processing steps in large scale slaughterhouse. 30% of fecal materials collected from incoming birds, 60% of air-chilled carcasses, 70% on carcasses at the post-spray wash site and up to 80% after cold storage carcasses were positive for *Salmonella*, indicating that cross-contamination occurred (Carramiñana, Yangüela et al. 1997). Another study also confirms the occurance of cross-contamination occurrence during slaughtering process; in which examined the presence of *Salmonella* in 400 chickens during different processing steps, 6% of the samples were positive for *Salmonella* after stunning, 24% were positive after evisceration, up to 52% were positive before chilling and 13% were positive after chilling (Mikalajczk and Radkowski 2002). The same condition could apply for the presence of Proteus. 15 % of the samples from small scale were positive compared to 12 % of the large scale. No significant differences in the percentage of positive *Proteus* were observed between the two types of facilities

Our findings showed that 42.5 % of the samples from small scale were *S. aureus* positive compared to 58 % of the large scale in our study. No significant differences observed between the two types of facilities (P>0.05). Similar to our findings, it has been shown that 65% of the tested samples on marketed poultry meat were positive to *S aureus* in Iran (Javadi and Safarmashaei 2011), while 73% of 45 chickens from two large scale slaughterhouses in Sweden were positive (Ternstom and Molin 1987). *S. aureus* is pathogenic to human and chicken, the

reason of the high presence of *S. aureus* in large scale slaughterhouses could be a large number of workers, poor personal hygiene, the technique of opening the abdomen with a technique of the hand evisceration and infrequent handwashing (Cohen, Ennaji et al. 2007).

In this study, all tested samples were negative for the presence of *Cl. perfringes* and *L. monocytogens* in both small and large scale slaughterhouses. In contrast, our results showed significant differences in the presence of *Campylobacter* between small scale (20%) compared to the large scale (6%) ($P < 0.05$). The Palestinian standards for fresh chilled chicken require a devoid of the product completely from *L. monocytogens* as a microbial requirement for the validity of fresh chilled chicken. This was achieved in both scales according to our study results (PSI 2013). *L. monocytogen* enter the food processing facility through the environment, or contaminated raw materials. Another study has found *L. monocytogenes* in 32% of meat samples from a total of 2242 samples from 12 food processing environments (FPEs). These 12 (FPEs) were divided to 2 groups, six of them were contaminated and the other six were assumed to be uncontaminated based on the *L. monocytogenes* occurrence. These results have also shown that a consistent cross-contamination risk exists and demonstrate that *L. monocytogenes* was common colonizers of FPEs in the European processing facilities sampled (Muhterem-Uyar, Dalmasso et al. 2015). In another study, 83% presence of *Cl. perfringes* in tested samples of marketed poultry meat (Javadi and Safarmashaei 2011), while *Cl. perfringens* was not detected in a study applied on 45 chickens from two large scale slaughterhouses in Sweden (Ternstom and Molin 1987). Another study by Denis et al., 2001 has shown a 5.6% presence of *campylobacter* of slaughterhouse samples and 17.5% of supermarket samples, his study found that *Campylobacter* contamination affects all stages of poultry production and processing significantly, while there was no significant difference for any type of samples between the molecular and conventional

technique for *Campylobacter* detection(Denis, Refrégier-Petton et al. 2001). In contrast, Chrystal et al., 2008 isolated *Campylobacter* from 163 tested carcass samples, 44.8% of collected carcass rinse samples, and 12.3% from weep water samples were positive for *Campylobacter* presence (Chrystal, Hargraves et al. 2008), and 13% presence of *Campylobacter jejuni* were detected from 45 chickens from two large scale slaughterhouses in Sweden (Ternstom and Molin 1987).

Our study has shown that changing of scalding water after every batch has decreased the presence of *E. coli* and *Campylobacter*, and also increased the presence of *S.aureus*. Using of chemical materials during washing before and during chilling and also in the cleaning process has decreased the presence of *E. coli*, *Salmonella,* and *Campylobacter*. It's necessary to reduce the microbial loads on carcasses by additional control intervention applications such as using decontamination treatments. Treatments applied to poultry carcasses include water, steam and chemical materials (Lactic or acetic acid, chlorine-based compounds, and trisodium phosphate). This resulted in overall microbial reductions of 0.6–3.8 log units. On the other hand, In the presence of organic materials, It's noticed that antimicrobial activity of some chemicals (e.g., chlorine compounds) is reduced (Buncic and Sofos 2012). A study depending on using a marker organism in poultry processing for identification of cross-contamination sites was designed by (Mead, Hudson et al. 1994), They have used a non-pathogenic, readily identifiable marker organism (nalidixic acid-resistant strain of *E. coli K12*) on a selected slaughtering steps to determine sites of cross-contamination in poultry processing procedures and to evaluate possible potential control measures. They found that cross contamination source was higher before scalding according to organisms inoculated on the carcass outer surface than those inoculated internally through the cloaca (Mead, Hudson et al. 1994). It has also shown that transport vehicles and crates are potential sources of contamination between batches of birds and between

farms. Attention should be given to avoid any unnecessary contact between carcasses and soiled surfaces during processing. As a result, using chlorine to control microbial contamination of carcasses and equipment at his study was not wholly successful(Mead, Hudson et al. 1994). Another study applied on 15 poultry flocks contaminated with *Campylobacter* has shown that processing and using of chlorinated-water sprays reduced the number of *Campylobacter* between 10-1000 fold. *Campylobacter* on the packaged carcasses processed with chlorine was significantly lower than the number of *Campylobacter* on the flocks before slaughtering (Mead, Hudson et al. 1995). Another study showed that the addition of 0.1% acetic acid to the scalding water drastically reduced the presence of *Salmonella* and *Campylobacter*. This reduction is thought to be via reducing cross-contamination and dissemination of *Salmonella* and *Campylobacter* in the subsequent processing steps since scalding is one of the first steps in poultry processing (Okrend, Johnston et al. 1986). On the other hand, (Humphrey, Lanning et al. 1981) found that adjustment of the pH of chicken scald tank water to 9.0 ± 0.2 lowered the destruction time at 52C during scalding process of a strain of *Salmonella typhimurium* from 34.5 to 1.25 min, and it's also reduced the TVC (Humphrey, Lanning et al. 1981). In addition, sodium carbonate was as effective as sodium hydroxide in increasing the death rate of *Salmonella typhimurium* and would appear to be a suitable alternative (Humphrey, Lanning et al. 1981). Another study in the USA was established by (Kemp, Aldrich et al. 2001) to determine the effectiveness of replacement of the offline reprocessing system with continuous online processing [COP]. the offline reprocessing system is a standard poultry processing method depending on the physical separation of carcasses, water washes, chemical disinfection (up to 50 ppm chlorine), and chilling. On the other hand, continuous online processing [COP] rely on using acidified sodium chlorite (ASC) as a main disinfectant, ASC is an antimicrobial agent

approved by the U.S. Food and Drug Administration (USFDA) for the treatment of processed poultry, red meats (beef, pork, and sheep), seafood, and fruits and vegetables. ASC is an effective inhibitor of E. coli on poultry carcasses when used as a spray or dip application at 1,200 ppm sodium chlorite. COP depending on adding a spray cabinet on the processing line just after the carcass washing station and immediately prior to the chiller. Fecal- and ingesta-contaminated carcasses were then permitted to remain online to transit through the inside outside- bird-washer (IOBW), then the ASC spray cabinet, before, finally dropping off into the chiller. The microbiological quality of fecally contaminated carcasses was found to be significantly better following COP treatment, in which E. coli was 0.59 log10 CFU/ml and *Salmonella* incidence was 10.0%, while when following standard offline reprocessing *E. coli* was 2.37 log10 CFU/ml and *Salmonella* incidence was 31.6%. COP also significantly reduced the residual titers of *Campylobacter*, residual titers reduced from 1.14 log10 CFU/ml (49.1% incidence) following COP to 2.89 log10 CFU/ml (73.2% incidence) in carcasses that following offline reprocessing. These results of this study support that the combined use of an inside-outside-bird-washer for the removal of visible contamination and an online ASC spray system to reduce microbial levels in commercially processed poultry is better than using offline reprocessing (Kemp, Aldrich et al. 2001). By comparing the two chilling methods, air and immersion chilling process, both of them significantly reduce bacterial concentrations on the carcasses according to (Huezo, Northcutt et al. 2007) study, and there was no difference observed in the bacterial concentrations between the two chilling methods, both chilling methods could obtain a reduction up to 90% in the concentrations of *E. coli* and *Campylobacter*, and both were microbiologically comparable without chemical intervention (Huezo, Northcutt et al. 2007). Another study by (Rosenquist, Sommer et al. 2006) supports that both air chilling and

water chilling caused similar and significant reductions in the bacterial count. However, an additional reduction of bacterial count in the packed frozen chickens due to freezing operation after water chilling, while the bacterial count remained at the same level after air chilling (Rosenquist, Sommer et al. 2006). In contrast, (Abu-Ruwaida, Sawaya et al. 1994) found that microbial levels varied during processing of Poultry in a Modern Commercial Slaughterhouse in Kuwait, Microbial levels did not change during evisceration, and Spray washing after evisceration did not reduce levels of bacteria, The highest levels were detected after scalding and defeathering and no substantial change occurred in bacteria levels during air-chilling, packaging and cold-storage. The final product was heavily contaminated and *Salmonella* was present in all birds examined (Abu-Ruwaida, Sawaya et al. 1994). Other study by (Lues, Theron et al. 2007) has shown that a higher presence and TVC of microorganisms was found in the receiving, killing and defeathering areas, whereas TVC and presence of microorganisms decreased in the evisceration, air chilling, packaging, and dispatch areas. This indicate the importance of controlling microbial levels before processing begin to prevent the spread of organisms downstream and reduce the risk of cross contamination (Lues, Theron et al. 2007). This also was supported by other study that contamination of the slaughter line with *Salmonella* leads to carcass contamination. Salmonella-free flock became contaminated during slaughtering with the same strains of Salmonella isolated previously from a contaminated slaughter line before beginning of slaughtering process (Rasschaert, Houf et al. 2007).

Conclusion

The presence of *Salmonella*, *Campylobacter*, *Cl. perfringens*, *L. monocytogenes*, *E. coli*, *S. aureus,* and *Proteus* in poultry final products represents an internationally human health concern since they are certain important zoonotic pathogens. Bacterial contamination is also a concern for shelf life in meat production. This comparative study applied on both small and large scale slaughterhouses in Palestine to investigate the presence of these microorganisms in the final product of both scales, and to determine total bacterial viable count and to evaluate the risk factors of bacterial contamination in the two processing methods. Although there is a huge difference in the processing procedures between both scales, but there is no significant difference between them. The results were not satisfied in both scales, It was expected that large scale slaughterhouse will be significantly lower in all the results from the small scale since as they are more hygienic, sterilized and automated. Potential cross-contamination and recontamination in the large scale slaughterhouses, poor sanitation practices, poor equipment design, and deficient of ingredients controlling explain the high presence and prevalence of different bacteria's inoculated from this scale.

Recommendations

This study highlights the need to reinforce industry preventive control measures. The outcome of this study should be used as a guide for the Palestinian customers, decision-makers and Public Health Authorities about the healthy and safe of poultry products. The widely consuming of unsafe poultry products in Palestine extent a real threat. This is the first study in Palestine concerned in the level and type of bacterial contamination in each processing method. Real preventative measurements should be taken in both large and small scale slaughterhouses. Hygiene education programs should be installed to raise consumer awareness of the risks of cross-contamination in the home and their role in its prevention. Slaughterhouse grading system should be designed and monitored by competent authorities. Developing the infrastructures, presence of diagnostic tools in slaughterhouse own laboratory and data references for all the procedures, and presence of veterinarian and health inspection all the time for health professions with help of quality control manager will lead to the best results with less possible bacterial contamination.

Indexes

Research Questionnaire

Research Questionnaire

"Level of microbial contamination in slaughterhouses compared to traditional chicken slaughter stores"

Husam Al-Sayyed

All information contained in this questionnaire is strictly confidential and will be used only for the purposes of research.

This Questionnaire contains 3 parts:

1- Basic information about the facility.

2- Qualitative questions.

3- Quantitative questions.

- ## Basic information

1. Address (City): Click here to enter text.

- ## Qualitative questions

#	Question	Yes	No	Notes
1st Part : legal and registration issues				
1	Is the facility has been registered in the governmental authority?			
2	Does the facility contain a veterinarian doctor for supervision the whole process?			
3	Does the facility hold a PSI certificate?			
4	Does the facility hold a quality certificate from 3rd party body? Ex: ISO 9001, ISO 22001, HACCP…etc.			
5	Does the facility contain its own laboratory to do the tests for raw material and finisl			

	goods?			
6	Are the live chickens being inspected and certified by any governmental authority be slaughtering?			
7	Does the veterinarian doctor make an inspection for live chickens before slaughterin			
8	Had the live chickens been grown in the company's own farms?			
9	Is there any record for the medications which were given to the live chickens?			
10	Were all employees tested in health center before working in the facility?			
2nd Part : facility infrastructure and departments				
1	Does the facility connect with municipality water supply?			
2	Does the facility contain a standby electricity generator?			
3	Does the facility connect with municipality sewage system?			
4	Does the facility contain a separate area for the reception of live chickens?			
5	Does the facility contain a separate area for the slaughtering process?			
6	Does the floor of the slaughtering area good maintained to prevent stagnant water an to be stuck?			
7	Does the facility contain a separate area for the storage of final products??			
8	Does area for the storage of final products has a cooling system?			
9	Does the cooling system contain an alarm in case of temperature fluctuation?			
10	Does the facility contain a separate area to deal with the customers?			
11	Does the slaughtering area need an authorization to enter in?			
12	Does the facility contain an air filtration system?			
13	Does the facility contain a pest control system?			
14	Does the facility make a contract with 3rd party body for pest control service?			
3rd Part : Slaughtering process				
1	Are the live chickens being fasted before slaughtering?			
2	Are the live chickens being stunned before slaughtering?			
3	Are the slaughtered chickens being fully bleeding?			
4	Do you change the scalding water after every batch?			
5	Do you inspect the chicken after evisceration?			
6	Do you see any marks for viscera laceration in the evisceration process?			
7	Do you wash the carcass before the chilling process?			
8	Do you use any chemical material during the washing?			
9	Do you do the water chilling process?			
10	Do you use any chemical material during the water chilling process?			
11	In case of using chemical material, do you test the concentration of this chemical?			
12	Do you measure the temperature after chilling process?			
13	Do you see any marks for feathers or non-edible parts after the chilling process?			
14	Do you take samples during the process for laboratory test?			

4th Part : Manufacturing practice and cleaning				
1	Do all employees commit in good manufacturing practice? (gloves, cap, mask, no food inside the production, no smoking…etc)			
2	Are all employees being trained and aware about quality and GMP?			
3	Do you do the cleaning after every batch?			
4	Do you have a clear cleaning process and all employees follow it?			
5	Do you use any detergent in the cleaning process?			
6	Do you use any antiseptic in the cleaning process?			
7	Do you use a hot water in the cleaning process?			
8	Do you spend more than 15 minutes in the cleaning?			
9	Do you have separate teams before and after the chilling process?			
10	Is there any chance for these teams to be mixed?			
11	Do you differentiate between these teams by special dress or marks?			
12	Do you take a swab test for the facility? Floor , roof..etc?			
13	Do you clean the tools and equipments after every production batch?			
14	Is there any chance for these tools or equipments to be used in different areas or departments?			

- **Quantitative questions**

#	Question	Answer		
1	Facility space	☐< 50 m2	☐< 500 m2	☐> 500 m2
2	Number of working personnel	☐< 10	☐< 50	☐> 50
3	Working hours	☐one shift	☐2 shifts	☐>2 shifts
4	Daily production capacity	☐< 500 chicken	☐< 2000 chicke	☐> 2000 chicke

Thank you for your kind cooperation!

Palesteinian standards for fresh chilled chicken

مواصفة فلسطينية

م ف ٣١٤-٢٠٠٣

الطبعة الثانية

حلت محل ٣١٤-١٩٩٩

الدجاج الطازج المبرد

Fresh chilled chicken

المحتويات

البند	عنوان البند	صفحة
١-	المجال	٢
٢-	المراجع التكميلية	٢
٣-	التعاريف	٢
٤-	التعبئة والتخزين والنقل	٢
٥-	وسم الإنتاج (بطاقة البيان)	٢
٦-	الاشتراطات العامة	٣
٧-	الاشتراطات التركيبية	٣
٨-	الاشتراطات الصحية	٣
٩-	الاشتراطات الميكروبية	٤
١٠-	المحتوى	٤
١١-	طرق الفحص	٤
١٢-	اصطلاحات	٤
١٣-	المراجع	٤

١- المجال

تحدد هذه المواصفة الاشتراطات التي يجب توفرها في لحوم الدجاج الطازج المبرد.

٢- المراجع التكميلية

٢-١ المواصفة الفلسطينية ١٥٧ بأجزائها الخاصة بفحص اللحوم ومنتجاتها.

٢-٢ المواصفة الفلسطينية ٢١ الخاصة بالانحراف في اوزان وحجوم المنتوجات الغذائية المغلفة والمعدة للبيع.

٢-٣ المواصفة الفلسطينية ٥٩ الخاصة بمدة صلاحية المواد الغذائية.

٢-٤ المواصفة الفلسطينية ١٣٥ الخاصة ببطاقة بيان المنتجات الغذائية المعبأة.

٢-٥ المواصفة الفلسطينية ٢٤٣ الخاصة بالدجاج المجمد.

٣- التعاريف

٣-١ لحوم الدجاج: هي الأجزاء الصالحة للاستهلاك الآدمي والناتجة من ذبح إناث وذكور طيور الدجاج.

٤- التعبئة والتخزين والنقل

٤-١ التعبئة

يجب أن تحافظ مواد التعبئة والتغليف على اللحم من التلوث أثناء النقل والتداول والتخزين وان تكون مطابقة للمواصفات الفلسطينية وفي حال عدم وجودها يجب أن تطابق المواصفات العالمية حديثة الاصدار مثل FDA, CODEX.

٤-٢ التخزين والنقل

٤-٢-١ يجب الالتزام بجميع الشروط والقواعد الصحية في حفظ وتخزين لحوم الدجاج لمنع فسادها وتلوثها.

٤-٢-٢ يجب أن تخزن لحوم الدجاج عند درجة حرارة لا تزيد عن ٤ °م ولا تقل عن ٠ °م.

٤-٢-٣ في حالة الشحن والنقل يجب أن تكون وسائل النقل مزودة بثلاجات لا تزيد درجة حرارتها على ٦ °م وذلك قبل تحميلها بالدجاج المذبوح وان تستعمل طرق عملية مناسبة في التحميل والتنزيل حتى تقلل من ارتفاع درجة حرارة اللحوم ويجب أن يتم تطهير الثلاجات قبل نقل اللحوم.

٤-٢-٤ في حالة الثلاجات الكبيرة المركزية يجب الاحتفاظ بمسجل أوتوماتيكي لتسجيل درجة الحرارة أثناء فترة التخزين أو اخذ بيانات عن درجة الحرارة بصفة دورية وتسجيلها في سجل خاص بذلك.

٤-٢-٥ في حال عرض الدجاج الطازج المبرد للبيع في ثلاجة عرض يجب أن لا تزيد درجة حرارة اللحوم بها على ٤ °م.

٥- وسم الإنتاج (بطاقة البيان)

يجب أن تحتوي بطاقة البيان على البيانات التالية على كل غلاف وبخط واضح وغير قابل للمحو:

٥-١ اسم المنتج وعنوانه وعلامته التجارية إن وجدت.

٥-٢ تاريخ الذبح وانتهاء الصلاحية باليوم والشهر والسنة.

٥-٣ عبارة لحم دجاج طازج مبرد.

٥-٤ الوزن الصافي للوحدة المغلفة الطازجة المبردة.

٥-٥ يجب ان تكتب على العبوات عبارة مذبوحة طبقا للشريعة الإسلامية.

٥-٦ تعليمات الحفظ والتخزين.

٥-٧ التعليمات الأخرى المحددة في المواصفة الفلسطينية م ف ١٣٥.

٦- الاشتراطات العامة

٦-١ يجب أن يثبت أن الدجاج من مناطق خالية من الأوبئة ومن الإشعاع.

٦-٢ يجب أن يثبت عدم معاملتها بالهرمونات.

٦-٣ يجب ان لا تزيد نسب بقايا المضادات الحيوية في لحوم الدجاج عن ما هو محدد بالمواصفات الفلسطينية ان وجدت، وفي حال عدم وجودها يتم الرجوع الى المراجع العالمية حديثة الاصدار مثل FDA, CODEX.

٦-٤ يجب ان يخلو الدجاج قبل ذبحه من الامراض.

٦-٥ يجب أن تكون لحوم الدجاج وأجزاؤه ذات مظهر طبيعي خالية من علامات التلف والفساد والكدمات والقاذورات والروائح المنفرة والتزنخ.

٦-٦ يجب أن تتم عملية التنظيف الكامل وقطع الرأس والأرجل بعد الذبح مباشرة ثم إزالة الأحشاء الداخلية دون حدوث أي تلوث بأي جزء أو مادة من محتويات المرارة أو القوا نص أو المريء أو غيرها.

٦-٧ يجب أن تتم عملية الذبح طبقا لأحكام الشريعة الإسلامية وان تكون الأدوات المستخدمة فيها نظيفة وحادة وان تتم عملية النزف بصورة كاملة.

٦-٨ يجب أن تكون خالية من الريش العالق بالجسم.

٦-٩ يجب أن يتم ذبح الدجاج في مسالخ مرخص بها من قبل الجهات الرسمية.

٦-١٠ يجوز تجزئة الدواجن بعد ذبحها.

٦-١١ يجب أن تجرى عملية التبريد مباشرة وخلال مدة لا تزيد على ساعة واحدة من ذبحه وتجهيزه في غرف التبريد ولمدة لا تقل عن ١٢ ساعة بحيث لا تزيد درجة حرارة اللحم عن ٤°م.

٦-١٢ يمنع تجميد الدجاج الطازج المبرد ويمنع عرضه للبيع في المجمدات (الفريزرات) بل يجب عرضه للبيع في ثلاجات عرض تتراوح درجة حرارتها ما بين (٠-٤)°م منذ استلامه وحتى انتهاء مدة صلاحيته.

٦-١٣ في حال ختم الدجاج، يجب استخدام الاحبار المطابقة للمواصفات الفلسطينية ان وجدت، وفي حال عدم وجودها يتم الرجوع الى المراجع العالمية حديثة الاصدار مثل FDA, CODEX

٧- الاشتراطات التركيبية

يجب أن تتوفر في لحوم الدجاج الطازج المبرد الاشتراطات التالية:

٧-١ يجب ان لا تزيد نسبة السائل المنفصل عن ١٪ بعد تعرضه لدرجة حرارة ١٠°م لمدة ٣ ساعات.

٧-٢ يجب ان لا تزيد نسبة المواد النيتروجينية الكلية الطيارة (TVB-N) عن ٥٠ ملغم /١٠٠غرام مقدرة كنيتروجين.

٧-٣ يجب ان لا تزيد نسبة الثيوباربتيوريك مالونالدهيد عن ٢,٤ ملغم.

٧-٤ يجب ان لا تتعدى نسبة الملوثات والمعادن الثقيلة عن الحدود المسموح بها دوليا.

٧-٥ يجب ان تكون بقايا العقاقير البيطرية والهرمونات وما شابهها عن الحدود المسموح بها دوليا ضمن مراجع Codex.

٨- الاشتراطات الصحية

يجب أن تتوفر الاشتراطات الصحية التالية بالمنتج النهائي:

٨-١ يجب أن يتم إنتاج وتعبئة اللحوم في ظروف صحية سليمة طبقا للمواصفات الفلسطينية الخاصة بذلك.

٨-٢ يجب أن تخلو من الطفيليات وأطوارها وافرازاتها التي تسبب ضررا بصحة المستهلك.

٨-٣ يجب أن يكون السطح خاليا من النموات الفطرية والمواد اللزجة.

٩- الاشتراطات الميكروبية

يجب أن تتوفر الاشتراطات الميكروبية التالية في لحوم الدجاج الطازج المبرد:

٩-١ يجب ألا يزيد العدد الكلي للأحياء الدقيقة ١×١٠^٦ خلية/غم.

٩-٢ يجب ألا يزيد عدد بكتيريا الكلوستريديوم برفرنجس على١٠٠ خلية/غم.

٩-٣ يجب أن تكون خالية من السالمونيلا في ٢٥ غرام عينة.

٩-٤ يجب الا يزيد العدد الكلي للكوليفورم عن ١٠٠ خلية/غم.

٩-٥ يجب الا يزيد الستافلوكوكس اوريس عن ١٠٠ خلية/غم.

٩-٦ يجب ان يخلو المنتوج من الليستيريا مونوسايتوجينيس.

١٠- المحتوى

يجب ان لا يزيد الانحراف في أوزان التعبئة عن ما هو محدد في المواصفة الفلسطينية م ف ٢١ تحت بند منتجات غير سهلة التعبئة.

١١- طرق الفحص

تجرى طرق الفحص حسب المواصفات الفلسطينية ان وجدت، وفي حال عدم وجودها يتم الرجوع الى المراجع العالمية حديثة الاصدار مثل FDA, CODEX.

١٢- اصطلاحات

لحم الدجاج	Chicken meat
السائل المنفصل	Dripped fluid
تبريد	Chilling
المواد النيتروجينية الكلية الطيارة	Total Volatile Bases Nitregen (TVB-N)

١٣- المراجع

١٣-١ المواصفة القياسية المصرية ١٦٥١-١٩٨٨ الخاصة بالدواجن المبردة.

١٣-٢ المواصفة القياسية الأردنية ٢٠٤-١٩٨١ الخاصة بالدجاج الطازج المبرد والمجمد.

DNA concentration and A260/A280 ratio of the tested samples

Table 18: DNA concentration and A260/A280 ratio of the tested samples

Sample numbers	DNA concentration (ng/ul)	A260/A280 ratio
1.	60.6	1.46
2.	28.9	1.45
3.	38	1.45
4.	44.3	1.48
5.	52.2	1.45
6.	50.2	1.45
7.	57.4	1.46
8.	91	1.50
9.	55.1	1.47
10.	92.6	1.53
11.	50.1	1.49
12.	84.3	1.47
13.	78.1	1.47
14.	60.5	1.47
15.	39	1.46
16.	41.9	1.44
17.	35.6	1.44
18.	25.2	1.42
19.	43.4	1.43
20.	96	1.47
21.	33.2	1.48
22.	63.5	1.50
23.	121.2	1.50
24.	75.5	1.48
25.	51.3	1.48
26.	73.3	1.46
27.	47.6	1.45
28.	100.9	1.45
29.	73	1.50
30.	23.5	1.49
31.	88	1.46
32.	149.7	1.46
33.	233.9	1.60
34.	130.3	1.54
35.	109.2	1.54
36.	99	1.52
37.	130.4	1.57
38.	125.3	1.7
39.	185.3	1.68
40.	147.4	1.64
41.	133.8	1.55
42.	166.8	1.58
43.	126	1.54

44.	182.7	1.71
45.	170.3	1.70
46.	76.1	1.58
47.	127.6	1.56
48.	112.8	1.57
49.	111.4	1.54
50.	99.4	1.52
51.	118.2	1.51
52.	110.6	1.54
53.	124.6	1.59
54.	101.5	1.56
55.	106.5	1.50
56.	97.6	1.61
57.	108.5	1.59
58.	93.4	1.58
59.	104.8	1.56
60.	107.9	1.53
61.	58	1.47
62.	52.1	1.44
63.	60.9	1.44
64.	58.8	1.44
65.	50.6	1.42
66.	43.7	1.36
67.	64.7	1.42
68.	72	1.34
69.	48.5	1.45
70.	72.7	1.45
71.	93	1.43
72.	105.1	1.45
73.	61.4	1.42
74.	75.6	1.44
75.	64	1.44
76.	84.8	1.44
77.	63.4	1.41
78.	67.2	1.43
79.	67.1	1.42
80.	72.8	1.44
81.	77.8	1.46
82.	58.3	1.42
83.	69.5	1.44
84.	72.9	1.43
85.	113.5	1.45
86.	90.5	1.45
87.	31.6	1.26
88.	80.9	1.45
89.	69.1	1.41
90.	61.3	1.43

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