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**Sudan University of Science and Technology
College of Graduate Studies**

**Bacterial Contamination in Broiler Carcasses in slaughter
houses , *E.coli* and *Salmonella spp* as Models in Jabalawlia
Khartoum State –Sudan**

التلوث البكتيري للحوم الدواجن في المجازر، الاشيريشيا القولونية والسالمونيلا
نموذجاً في جبل اولياء - ولاية الخرطوم- السودان

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الآية

قَالَ تَعَالَى:

﴿وَفَاكِهَةٍ مِّمَّا يَتَخَيَّرُونَ ﴿٢٠﴾ وَلَحْمِ طَيْرٍ مِّمَّا يَشْتَهُونَ ﴿٢١﴾﴾

الواقعة 20 - 21

Dedication

This thesis is dedicated to , my iron women ;

my mother , father ,

brothers and sister who

encouraged and supported me in my life ,

and to my friends .

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Firstly thanks are given and praised to ALLAH , for giving me the energy and patient to finish this work .

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ABSTRACT

This study was performed in Khartoum State between April to June 2017. Two types of slaughtering houses, namely; automatic and manual were chosen to evaluate the bacterial count in broiler carcasses in three steps during slaughtering (after slaughtering, scalding and chilling), at three sites in broiler carcasses namely; leg, neck and brisket and from worker's hands during slaughtering. A total of 70 swab samples were collected; 35 samples from the automatic slaughterhouse; 5 samples from broiler after slaughtering directly. 15 samples during scalding (4 samples from leg, 4 from neck, 4 from brisket 3 samples from workers hands). 15 sample during chilling (4 sample from leg, 4 from neck, 4 from brisket and 3 samples from worker's hands). 35 samples from manual slaughterhouse; 5 samples from broiler after slaughtering directly. 15 samples during scalding (4 samples from leg, 4 from neck, 4 from brisket 3 samples from worker's hands). 15 sample during chilling (4 sample from leg, 4 from neck, 4 from brisket and 3 samples from worker's hands). All swab samples were transported in peptone water and examined in laboratory using blood agar media to count the colonies. The total Viable Count (TVC) of bacteria was low in brisket and worker's hand (3 CFU) during chilling in automatic slaughtering, and high in leg (205 CFU) during scalding in manual slaughtering. Samples were cultured in salmonella/ shigella agar (S.S agar) to isolate salmonella and cultured in Mac Conkey's agar to isolate *E.coli*. Primary tests were performed after isolation using gram stain, catalase, oxidase and oxidation fermentation tests to identify the bacteria, finally using API20E as a secondary test for identification of *E.coli*.

Statistical Analysis using SPSS version 18 Chi square the result showed that difference between sites in broiler carcasses . after slaughter steps the higher *E.coli* found in automatic slaughtering than other steps in the two type of slaughtering .

Result of bacterial identification revealed no *Salmonella* (0%) in all samples , while *E.coli* was isolated from three(3) samples from automatic slaughterhouses and (8) positive sample from manual slaughterhouse .A total of eleven (11) positive *E.coli* samples from worker's hands were 2 (2.85%) , while 9 (12.85%) positive *E.coli* sample were isolated from other chicken parts.

ملخص الاطروحة:

هذه الدراسة اجريت في ولاية الخرطوم (محلية جبل اولياء) في الفترة من ابريل و يونيو ٢٠١٧ م .

تم جمع عينات من نوعين من مجازر الدجاج اللاحم الاولي مجزر الي والثانية الذبح يدويا لتقييم عدد البكتيريا في اللحوم المنتجة ، و تم ذلك في ثلاث مراحل اثناء الذبح (بعد الذبح ، عند نزع الريش وفي مرحلة التبريد) من ثلاث مناطق في جسم الفرخة (الارجل ، الرقبه و منطقة الصدر) و من ايدي العمال خلال الذبح في مرحلتى نزع الريش و التبريد فقط .

تم جمع عدد ٧٠ مسحه : ٣٥ مسحه من المجزر الالى ٥ مسحات من الدجاج بعد الذبح مباشرة . ١٥ مسحه اثناء نزع الريش و هي كالاتي (٤ مسحات من الارجل ، ٤ من الرقبه ، ٤ من الصدر و ٣ من ايادي العمال) ، و ١٥ مسحه خلال مرحلة التبريد (٤ من الارجل ، ٤ من الرقبه ، ٤ من الصدر و ٣ من ايادي العمال). تم نقل العينات في ماء البيبتون الى المعمل و تمت زراعتها في ابيغار ميديا المدمم لعد المستعمرات البكتيرييه، وجد ان العد الحيوى الكلى للباكتيريا اقل عددا في المسحات من صدر الدجاج و ايدي العمال (٣) في مرحلة التبريد في المجزر الالى ، و اعلى عدد في الارجل (٢٠٥) في مرحلة نزع الريش في المجزر اليدوى . تم زرع المسحات في الايغار المغذى لعزل الباكثيريا و زراعتها في (ايس ايس ايقار ميديا) سالمونيلا شايقيلا ميديا لعزل السالمونلا، و التزريع في ماكونكي ايقار ميديا لعزل باكتيريا الاي كولاي.

تم اجراء الاختبارات الاوليه بعد عزل الباكثيريا باستخدام صبغة جرام لمعرفة البكتيريا ، اختبار الاوكسيديز ، الكتلينز ، اختبار الاكسدة والتخمر للتعرف على الباكثيريا . و اخيرا اختبار (اي بي اي ٢٠ اي) للتعرف على الايشيريشيا القولونيه كاختبار ثانوي.

التحليل الاحصائي باستخدام (حزمة التحليل الاحصايى الاجتماعى) الاصدار ١٨ اوضح وجود اختلاف في النتائج بين مواقع اخذ العينات في جسم الفرخة ، و اظهر ارتفاع باكتيريا الاي كولاي في مرحلة بعد الذبح مباشرة في المجزر الالى مقارنة ببقية المراحل في كلا انواع المجزرين .

اظهرت نتائج العينات خلوها من بكتيريا السالمونيلا، اما باكتيريا الي كولاي فقد وجدت ثلاثة (٣) عينات ايجابية في المجزر الالي وثمانية (٨) (١١.٤%) عينات ايجابية في المجزر اليدوي، العدد الكلى لوجود الايشيريشيا القولونية هي احدى عشر عينة (١١) (١٥.٧%) في كل من النوعين للمجازر.

عدد العينات التي وجدت بها الايشيريشيا القولونية في ايدى العمال هي ٢ (٢.٨٥%) عينة مقارنة بعدد العينات من اجزاء الدجاج المذبوح وهي ٩ (١٢.٨٥%) عينات .

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INTRODUCTION

Poultry meat and eggs are a leading source of animal protein for human consumption in many countries because there is little or no religious and or cultural restrictions in the consumption of these products. (Ghana Poultry Report Annual, 2013). Food contamination refers to the presence in food harmful chemicals and microorganisms which can causes consumer illness . other define it as , foods that are spoiled or tainted because they either contain micro-organism such as bacteria , parasites , or toxic substances , that make them unfit for consumption . contamination is also defined as the presence of substances that make food unfit for human consumption . Examples include bacteria, toxic chemicals, carcinogens, teratogens and radioactive material(Medical–dictionary) . Food contamination is a complex subject including pathogenic contamination such as that caused by bacteria , viruses and parasites ([https://Askkaren . com](https://Askkaren.com) (2009) . Broiler meat consumption is known as one of the main sources of food-borne infections in humans (Fitzgerald *et al.*, 2001). Processing of poultry products requires a severe microbiological quality control, considering that they are the most accessible protein sources. The contamination control during slaughter and processing has been identified as an ultimate requirement in order to detect the prevalence of pathogenic micro-organisms in poultry products. (Abu-Ruwaida *et al* ,1994). Production and consumption of poultry meat and poultry meat products show an upward trend. This, of course, requires adequate control and inspection both during poultry rearing and in slaughterhouses, processing plants and shops. Consumers are also a link in the chain of food-borne human diseases, because of the way they store and cook poultry meat and meat products. (Kozacinski, *et al* , (2006) . It is first

important to understand what the potential hazards are when it comes to food safety. Food contamination refers to food that has been corrupted with another substance – either physical, biological or chemical. (Australian Institute 2016)

The poultry industry has implemented several control programs, and these programs have improved the overall broiler carcass quality through processing (James, *et al* ,1992., and Lillard, 1990.).

Objectives :-

- 1- To determine the total viable bacterial load in manual and automatic broilers slaughterhouses .
- 2- To isolate and identify *Salmonella Spp* and *E.coli* from broiler carcasses in manual and automatic slaughter house .

Chapter One

Literature Review

1.1 Bacterial contamination of chicken carcasses

The level of *Enterobacteriaceae* in poultry carcasses can be routinely used as an indicator of improper hygiene methods during processing and incorrect storage conditions, which can lead to the proliferation of pathogens, such as *Salmonella*, and toxin production (Roberts *et al.*, 1995). *Campylobacter spp.* and *Escherichia coli* O157:H7, have been in a number of food borne illnesses. The contamination caused by these bacteria in the slaughterhouse occurs during processing of live animals into meat where the routine veterinary inspection procedures cannot detect the presence of these bacteria on meat (Nouichi and Hamdi , 2009) , Göksoy , *et al*, 2004) and Govender, 2014) . Dickson and Anderson ,(1992) stated that broilers arriving to the poultry slaughterhouse for processing are generally highly contaminated with bacteria , such as *Salmonella* spp., *Staphylococcus aureus* , *Listeria monocytogens* , especially with potential human pathogenic bacteria, such as Coliform and *Salmonella*. It is also reported that slaughtering process results in surface tissue contamination with counts of micro-organisms ranging from 10^2 to 10^4 colony forming units(cfu) /cm² .

Salmonellosis is a major public health concern and continues to have a serious economic impact on the U.S. poultry industry (Morales and *Mc Dowell* ,1999) . *Salmonella* food poisoning in humans was often associated with the consumption of Pathogenic poultry products (Coyle, *et al* ,1988), (Hogue, *et al* , 1997) , (Lin, *et al* , 1988) , and (Olsen, *et al* ,2000). The poultry industry has implemented several control programs, and these

programs have improved the overall broiler carcass quality through processing (James, *et al* , 1992) , (Lillard, 1989) ,(Lillard, 1990) . Contamination of broiler meat with *Salmonella spp* , *Escherichia coli* and *Staphylococcus spp.* was evident in many studies. Ahmed , (2014) assessed the measures of poultry meat safety in one of the Khartoum State slaughterhouses with the aim to detect the bacterial load and types of bacteria on the carcasses of broiler by applying the principles of HACCP. The contaminating bacteria isolated in the study were *E. coli*, *Staphylococcus spp.* and *Salmonella spp.* The researcher recommended that appropriate methods of HACCP should be applied during slaughtering operations so as to reduce the occurrence of these microorganisms. Another study conducted by Omer, and Abdelrahman, (2015) on *Salmonella spp.* and *Escherichia coli* contamination in poultry meat carcasses at an automatic slaughterhouse in Khartoum State concluded that the *Salmonella spp.* and *Escherichia coli* were predominant in slaughterhouses processing and this affected safety and quality of poultry meat. They recommended that the right application of HACCP will greatly reduce the bacterial contamination. The contaminating bacteria isolated in different slaughterhouses and their contamination level was studied by different researchers. Mohamed-Noor, *et al* , (2012) studied microbial contamination of broilers in modern abattoirs in Khartoum State in 9 processing steps(PSs). The bacterial species isolated from different PSs were *Staphylococcus aureus*, *Staphylococcus albus*, *Escherichia coli* and *Salmonella spp* . Their results showed variability of microbial counts during processing of broilers carcasses that may cause public hazard. Food borne infections and illness is a serious worldwide health problem associated with economic losses. Poultry is an important part of the animal food market and production is increasing to satisfy public demand world-wide (Bryan 1980). The consumption of poultry meat has increased worldwide within the last decades (FAO,1993; McNamara; 1997; Mead 1997).Meat is considered as an

important source of proteins to man and is the most Perishable of all important foods because of its rich nutrients that supports microbial growth (Magnus, 1981; Ukut *et al.*,2010) . Epidemiological data suggested that contaminated products of animal origin especially poultry contribute significantly to food borne diseases. Reduction of raw poultry contamination levels have a large impact on reducing the incidence of illness (Keener *et al*, 2004). Each year, millions of people worldwide suffer from food-borne diseases (WHO,2000), and illness resulting from the consumption of contaminated foods has become one of the most widespread public health problems in contemporary society (Notermans *et al.*, 1995).

According to FAO (1988), it was stated that for human diet poultry meat had been considered a valuable source of protein. Nevertheless, the composition as substrate was claimed to provide the need for growth and multiplication of many genera of spoilage and disease producing bacteria (Jay, 1986). These varieties of bacteria might perpetuate if poultry meat was not properly prepared and hygienic practice were not followed (Jensen, 1954). The rearing of large numbers of table poultry for slaughtering at high throughput was also found to contribute in failure of reducing the bacterial contamination of carcasses (Price and Schweigert, 1971.). The significance of bacterial contamination in poultry meat was organoleptic changes or spoilage and human illness due to infection or intoxication. In this respect, they described meat as an ideal culture medium for many organisms. The reasons were its high moisture, richness nitrogenous protein, plentiful supplement with minerals and accessory growth factor, presence of fermentable carbohydrates and its favorable pH for most microorganisms.(Drewnaik *et al.*,1954) , they found that there was a buildup of bacteria on the skin of chicken during dressing and evisceration. They also found that the procedures after dressing which include washing with pressurized sprays of water decreases the bacteria present on the a skin of poultry .

However, broilers entering slaughter processing are highly contaminated by microorganisms, including food borne pathogens such as *Salmonella* and *Campylobacter* spp., and these pathogens tend to be disseminated in the processing (Mead *et al*, 1994). The micro-organisms were widely distributed over the carcasses under normal circumstances and were spread over the skin during scalding and defeathering and on the inner and outer surfaces during evisceration and further processing (Bailey *et al.*, 1987). The procedure for converting a live, healthy bird into a safe and wholesome poultry product provided many opportunities for micro-organisms to colonize on the surface of the carcasses (Bryan, 1980; Thomas and McMeekin., 1980; Roberts, 1982; Grau, 1986; Bailey *et al.*, 1987; Connor *et al.*, 1987; Banwart, 1989; Mead, 1989).

Food safety of poultry meat, egg and products remains a serious problem in many countries in the world. A Study by (FAO\WHO,2002) reported that food poisoning is one of the widespread health problem which was considered as one of the reducing economic productivity both in developed and under developed countries. Estimation of 76 million people in the United States experienced food poisoning infection causing 325,000 hospitalizations and 5,000 death (mead ,*et al*,1999) .

sually the steps practiced at different points in processing poultry meat are (FAO / WHO ,2002) :

1.2 Receiving area :

The inlet to the plant is normally designed in such a way that fluctuations in bird deliveries can be dealt with adequately. This is necessary since the processing capacity has a fixed maximum. At regular intervals. Birds are unloaded on to the holding areas , attached by their feet to a conveyor belt and transported to the slaughter area.

1 - Slaughtering and packing

The birds are suspended from the conveyor after which the following actions take place:

2- Stunning;

3- Killing and bleeding by cutting the jugular veins

- Collection of blood: The conveyor travels through a blood collection tunnel at a preselected travelling speed;

4- Scalding : To loosen the feathers, the birds are held in water of temperatures ranging from 50°C to 60°C;

5- Defeathering : Feathers are mechanically abraded from the scalded birds, usually by rotating rubber fingers. Removed feathers drop in underlying troughs;

6- Washing : The de feathered carcasses receive a spray wash prior to evisceration;

7- Opening of the carcass: by cutting manually;

- Inspection of the viscera

8- Evisceration: removal of head, feet and viscera;

a- Sorting of the viscera: to recover heart, liver and gizzard;

b- Final washing: to remove blood and to loosen tissues;

c- Chilling of the carcasses: in a water bath;

d- Draining:

e- Grading: weighting and packing; and

9- Chilling and freezing:

During slaughter most of the microorganisms are eliminated , but subsequent contamination is possible at any stage of the production process, from feather plucking , evisceration, and washing to storage by cooling or freezing. Microorganisms from the environment, equipment and operators' hands can contaminate meat (Mead, 1989; Živkovic, 2001) . the micro-flora of poultry is transferred from the primary production sites to production lines, and further, by subsequent contamination. Microflora of crude chicken meat is heterogeneous and originates from slaughtering premises, operators' hands, equipment and outfit, and water and air , Contamination with pathogenic bacteria, in particular *Salmonella*, plays an important role in the veterinary-sanitary control of meat. (Anonym 1996 , Fries 2002) .

1.3 Food Safety Management System (FSMS):

Hazard Analysis Critical Control Point (HACCP) : A system which identifies , evaluates , and control hazards which are significant for food safety (Goksoy , *etal* , 2004). Measures to reduce such contamination in the processing plant could also reduce subsequent cross-contamination in the kitchen and thus the risk to consumers. In attempting to identify and control those sites in poultry processing at which microbial transmission occurs, [Mead,*etal*,1994] .Good Hygienic Practices (GHPs) and Good Manufacturing Practices (GMPs) are prerequisites for the application of Hazard Analysis Critical Control Points (HACCP) system to food safety operations (Govender , 2014) . FSMS aims to prevent, eliminate or reduce the incidence and levels of pathogenic microorganisms which can compromise human health (Akinnibosun, *and Imade* , 2015). (Howlett , *et al* ,2010) stated that to develop a FSMS in slaughterhouses is to implement pre-requisites programs

i.e. GMP and GHP . Food borne infections and illness is a serious worldwide health problem associated with economic losses (Bryan 1980) , Broilers entering slaughter processing are highly contaminated by microorganisms, including food borne pathogens such as *Salmonella* and *Campylobacter spp*, and these pathogens tend to be disseminated in the processing (Mead *et al.*, 1994). The micro-organisms were widely distributed over the carcasses under normal circumstances and were spread over the skin during scalding and de-feathering and on the inner and outer surfaces during evisceration and further processing (Bailey *et al.*1987).

HACCP has become synonymous with food safety. It is a worldwide recognized systematic and preventive approach that addresses biological, chemical and physical hazard through anticipation and prevention, rather than through end product inspection and testing .For the HACCP system to be successfully implemented , the concept must be understood by food business owners and managers. Their commitment is crucial for the staff to effectively operate the system. A major role of governments and industry/trade associations in the developing countries is to provide adequate, accessible technical support including HACCP prerequisites for small and medium abattoirs as consultancy is rarely available , Hazard Analysis and Critical Control Points (HACCP) is a system of process control. It is designed to prevent the occurrence of problems by ensuring that controls are applied at any point in a food production system where hazardous or critical situations could occur. Hazards could include microbiological, chemical, or physical adulteration of food products (FAO/WHO 2004).

1-4 Handling of live birds :

- 1- Birds must be handled as little as possible up to the point of slaughter.
- 2- Birds must be unloaded from crates and slaughtered within two hours of arrival at the slaughter plant.
- 3- Birds must be unloaded from crates in a dimly lit room.
- 4- Birds must not be caught or carried by one leg.
- 5- Unloading of crates must take place as close as possible to the cones or shackle line to minimize carrying distance.
- 6- Inversion and suspension of birds must be minimized.
- 7- No bird can be inverted or suspended in such a manner as to cause it avoidable pain or suffering.
- 8- Birds must not be inverted and placed in cones or on shackles unless the personnel and means to slaughter them are immediately available and operational.
- 9- If birds are placed in cones these must be the correct size for the type of bird.
- 10- No chicken or duck can be inverted for more than 60 seconds before being stunned or killed.
- 11- No turkey or goose can be inverted for more than 120 seconds before being stunned or killed.

12 -Abuse or maltreatment of birds is prohibited. The following actions are prohibited and are considered by *Animal Welfare Approved* to constitute cruelty

A-Shackles ;

* Shackling of live birds is not carried out without consent from *Animal Welfare Approved* .If shackles are used they must be the correct size and width for the birds. If shackles are used birds must be hung by both legs

B-Stunning and slaughter

- Stunning must render birds immediately insensible to pain on the first attempt.

- Stunning must ensure birds remain insensible to pain until the bird dies due to slaughter or blood loss.

C- Bleeding

Once the bird has been rendered insensible it must be bled.

Birds must be bled within 10 seconds of stun.

1-5 Food borne Bacterial infections in human :

Bacteria are traditionally divided into the two groups: gram-positive and gram-negative, based on their Gram stain retention. These groups are often thought of as lineages, with gram-negative bacteria more closely related to one another than to gram-positive bacteria. While this is often true, the classification system breaks down in some cases. A given bacteria's Gram stain result, bacterial membrane organization, and lineage groupings do not always match up.-(*Gupta (December 1998)*);(*Gupta (2000)*) ; (*Desvaux et al*

, (April 2009). ;(Sutcliffe (October 2010). As such, the Gram stain cannot be reliably used to assess familial relationships of bacteria. That said, Gram staining does often give reliable information about the composition of the cell membrane, distinguishing between the presence or absence of an outer lipid membrane.(Gupta (December 1998) ;.(Gupta (August 1998).

The proteobacteria are a major group of Gram-negative bacteria, including *Escherichia coli*(*E.coli*), *Salmonella* ,*Shigella* ,and other *Enterobacteriaceae*, *Pseudomonas* ,*Moraxella*,*Helicobacter* ,*Stenotrophomonas*, *Bdellovibrio*, acetic acid bacteria, *Legionella etc.* and other bacteria. (FAO\ WHO (2002)

The microorganisms in different part of carcass ,carried out on food from animal origin, particularly poultry product, contribute significantly to food-borne disease in humans , during processing, a high proportion of this organisms will be removed and will result in reducing the incidence of illnesses but further contamination may occur at any stage of processing operation (Kabour 2011). Medically relevant gram-negative bacilli include a multitude of species. Some of them cause primarily respiratory problems (*Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*), primarily urinary problems (*Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*), and primarily gastrointestinal problems (*Helicobacter pylori* , *Salmonella enteritidis* , *Salmonella typhi*). Thermophilic *campylobacters*, especially *Camnpylobacter jejunii* are a major cause of acute gastrointestinal infection in man, with more than 38000 reported cases in the UK during 1992 . Poultry meat becomes contaminated with *campylobacters* because the live bird is frequently a symptomless intestinal carrier of the organisms and dissemination readily occurs during processing (Oosterom , et al , 1983.- Berndtson , et al,1992.). Rapid growth in consumer demand for poultry and poultry products over the last decade and increased international trade in these foods have focused attention on

objective measures of food safety and quality. There is no specific policy regarding slaughter, dressing and sale of poultry meat in India. As a result roadside slaughter in most unhygienic manner is prevalent in most of the cities, towns and villages of India. Although a very few modern poultry processing plants have been established, majority of the consumers purchase meat from the roadside shops or small retailers where chickens are being slaughtered and dressed in unhygienic condition in their presence (Das and Biswas, 2003). a Salmonellosis is a major public health concern and continues to have a serious economic impact on the U.S. poultry industry (Morales and McDwell 1999.)

1-6 Gram-negative bacteria:

Are a group of bacteria that do not retain the crystal violet stain used in the Gram staining method of bacterial differentiation.(Baron ,et al.1996)They are characterized by their cell envelopes , which are composed of a thin peptidoglycan cell wall sandwiched between an inner cytoplasmic cell membrane and a bacterial outer membrane.

Gram-negative bacteria are spread worldwide , in virtually all environments that support life. The gram-negative bacteria include the model organism *Escherichia coli*, as well as many pathogenic bacteria, such as *Pseudomonas aeruginosa* , *Neisseria gonorrhoeae* , *Chlamydia trachomatis* , and *Yersinia pestis* . Several classes of antibiotics target gram-negative bacteria specifically, including aminoglycosides and carbapenems.

Skin of poultry carcasses always exposed to high average rate of microorganisms, they can be pathogenic that cause food-borne illness as well as food spoilage . Microorganisms on the surface of carcasses can be analyzed in order to indicate the microbial quality , the level of hygiene in production

and handling and the correct maintenance of cold chain (Sandro and Arvanitoyannis,1999) .These systems present some advantages of traditional methods, and results obtained in study from eight slaughter houses suggested that HACCP systems can maintain or even improve food safety(Cates *et al*, 2001).

The contamination and or cross- contamination of carcasses, during slaughter process were demonstrated and results indicated presence of bacteria of potential public health significances (Doyle,*etal*, 1987) .Also dirty work as hands , clothes , equipments of slaughterhouse . acts as intermediated sources of contamination of meat (Gill, 1998; Gilmour et al., 2004). Also due to defeathering the microorganisms are widely distributed under normal circumstances and are spread over the skin during scalding and defeathering on inner and outer surfaces during evisceration of the further processing(Bailey *et al*, 1987) .

1-7 Bacteria associated with food borne disease :

1.7.1 *Salmonella spp* :

Salmonella spp are Gram-negative rods ,facultative anaerobic belong to Enterobacteriaceae Family . there are high importance in food safety , lead to severe intestinal infection in human can lead to death especial older people (WHO,2005 , Kleer 2004) .

Salmonella are not part of the normal intestinal micro flora of poultry, but are acquired from the farm environment via insects, rodents and birds. Feed is also an important source of salmonella through contamination of various components of the feed mix. There are many sources from which poultry may obtain Salmonella, the main sources being from cross- contamination during breeding, hatching and intensive rearing operations .(Jones *et al*, 1991) ; Silliker and Galois , 1986; Mead, 1989). *Salmonella sp.* species are

responsible for a variety of acute and chronic diseases in both poultry and humans. Contaminated poultry products are among the most important sources for food-borne outbreaks in humans. Isolations of *Salmonella* sp. are reported more often from poultry and poultry products than from any other animal species (Myint, 2004). causes typhoid fever, paratyphoid fever and food borne illness (Ryan and Ray, 2004). one of the commonest causes of *Salmonella* spp. infection reported in humans has been through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat (Panisello *et al.*, 2000). *Salmonella* spp contamination on 25% of chicken samples collected from retail outlets in Lohore city was reported by Ahmad *et al.*, (2013) . contamination of chicken sample with *salmonella* spp was reported as high as 60% in Portugal (Antunes *et al.*, 2003), 25% in England (Jorgensen *et al.*, 2002). A survey conducted (52.0 %)by Hassanein *et al.*, (2011) in Egypt .

1.7.2 E. coli :

Escherichia, a genus of family Enterobacteriaceae . Its described as a coliform of the intestinal tract of man and other animals from which it might be found in soil , water and many other places in nature. E.coli Certain serotypes of E.coli produce food-borne disease , It is a Gram negative bacteria an aerobic and(Gordon and Cowling 2003) and (Berg ,1996). E.coli known as pathogenic bacteria causing sever intestinal disease in human (kaper el at 2004) . most *E. coli* strains are non-pathogenic but pathogenic strains that cause gastrointestinal illness in humans and opportunistic ones that normally affect immune-compromised patients exists (Nataro and Kaper, 1998). Enterohemorrhagic *Escherichia coli* (EHEC) strains of *E. coli* contain specific virulence properties: lysogenic phage encoding one or more Shiga toxins (with or without a chromosomal pathogenicity island), and often an additional virulence plasmid. These strains are also referred to as Shiga-toxin

producing *E. coli*, or STEC. Most EHEC strains are serotype O157:H7 (Noël and Boedekar, 1997). ETEC (*enterotoxigenic E. coli*) strains are a major cause of secretory diarrhea in both humans and animals (Bern *et al.*, 1992). ETEC produce toxins which are heat-labile (LT) and/or heat-stable (STa and STb) that are also causing diarrhea (Bern *et al.*, 1992). *E. coli* has been isolated worldwide from poultry meat (Canton *et al.*, 2008; Adesiji *et al.*, 2011). In Nigeria, 16% have been isolated in Osogbo (Adesiji *et al.*, 2011), 48.4% in Morocco (Cohen *et al.*, 2007), a total number of 212 chicken samples taken to assess the prevalence of *E. coli* in Greater Washington, D.C., area in 2001, 82 (38.7%) yielded *E. coli*. (Zhao *et al.*, 2001).

1.7.3 Klebsiella

Klebsiella, a genus of the family Enterobacteriaceae. This genus is Gram-negative rods, aerobic and facultative anaerobic, non-motile, capsulated, catalase positive, oxidase negative and attacked sugars fermentative. This genus is among the infections due to miscellaneous micro-organisms. *Klebsiella* was the predominant flora in faecal samples from outbreak of poisoning involves 30 students (Riemann, 1969).

1.8 Gram positive bacteria:

1.8.1 *Listeria monocytogene*

Listeria monocytogenes, Gram positive is widely distributed in nature and the environment. These organisms are isolated from soil, vegetation and faeces of humans and animals, with poultry often being contaminated. Studies also indicated that 57% (20 of 35 samples) and 33% (17 of 51 samples) of market poultry, respectively, contained *L. monocytogenes*. *L. monocytogenes* can multiply at refrigeration temperatures. Data also suggests that *L. monocytogenes* is more heat resistant in meat than *Salmonella*. The necessity of proper hygiene procedures in handling, processing and

packaging of poultry is therefore emphasized). *L.monocytogenes* can multiply at refrigeration temperatures. Data also suggests that *L. monocytogenes* is more heat resistant in meat than *Salmonella*. (Terplan et al 1986)

1.8.2 *Bacillus cereus* :

Gram positive facultative anaerobic rod its widely spread , forming spores , cause two type of disease in human :a diarrhoea type due to the production of Enterotoxin in small intestine and an emetic type caused by ingestion of the toxin . Many are as important in the spoilage of many foods held above refrigerator temperature. *B.cereus* and *B.mesentericus* were species reported to be involved in food-poisoning. The characters of members of the genus are aerobic, (Wegschneider ,2004 ; Becker et al ,2005 ; EFSA,2003)

1.8.3 *Campylobacter spp* :

is more sensitive than many other organisms to the adverse effects of environmental conditions (drying, freezing and cold storage). For this reason, attention has been given to factors influencing the survival of campylobacter's in processing. Although freezing is harmful to *Campylobacter*, it does not eliminate this organism from poultry. Nevertheless, the contamination rate tends to be higher in fresh than in frozen carcasses. *Campylobacter spp.* are also more sensitive to chlorine than *E. coli*, but are not eliminated from poultry carcasses by immersion chilling in chlorinated water.

Campylobacter is widely spread in nature and is isolated from wild and domestic animals as well as from the environment. Poultry is a major reservoir of *Campylobacter.jejuni* . Many commercial poultry flocks appear to be symptomless carriers of *C. jejuni* , with up to 10⁷ .g-1 of gut content being demonstrated in the ileum and caeca of infected poultry and similar

levels in the feces (Genigeorgis et al., (1986) ; Mead, (1989); Zottola and Smith., (1990).

Some poultry flocks that are negative before slaughter will therefore become contaminated during processing. Campylobacter is micro aerophilic with a relative high minimum growth temperature (30°C) and there seems little likelihood of them multiplying in the processing plant or on the raw, processed product. The main problem in processing is that of cross-contamination (Zottola and Smith (1990) ; Smeltzer, (1981). Campylobacter was also isolated from air samples as well as equipment (Cunningham, 1987; Mead, 1989; Zottola and Smith., 1990).

1.8.4 *Staphylococcus* :

Staphylococcus , a genus of the family Micrococcaceae. It is commonly found in the nasal cavity and skin of man and certain other animals. The coagulasepositive members of the genus (e.g. *Staphylococcus aureus*). Food poisoning from poultry meat caused by *Staphylococcus aureus* is much less common than that due to salmonellas or *Clostridium perfringens* (Todd, 1980; Mead, 1982). *Staphylococcus* is important in relation to poultry meat, because it can produce enterotoxins which may cause food poisoning in humans (Notermans et al., 1982).

Evisceration and chilling are also processing stages which have been incriminated in contaminating carcasses with *Staphylococcus aureus* (Gibbs et al., 1978; Todd, 1980; Mead, 1982; Notermans et al., 1982; Mead, 1989).

1.9 Conception of HACCP System :

Hazard Analysis Critical Control Point (HACCP) :A system which are indentifies , evaluates , and control hazards which significant for food safety . (Ying Zhen (2011). Measures to reduce such contamination in the processing plant could also reduce subsequent cross-contamination in the kitchen and thus the risk to consumers. In attempting to identify and control those sites in poultry processing at which microbial transmission occurs, [MEAD et al ,1994] .

HACCP in poultry is extremely important because it involves the constant monitoring of all steps of the process , aiming the food safety of final product ,industries must implement this food safety program to serve both external and internal market (Jimenez et al., 2002, Mead 2004 ,Galhardo et al., 2006).

One the most important parameters intervening in bio security of slaughterhouses is cleaning and decontamination procedures that will depend on the hygienic statute of the slaughterhouse , but also quality of the poultry meat during slaughtering and packaging (Lillard 1990). The poultry industry has implemented several control programs, and these programs have improved the overall broiler carcass quality through processing (James, el at , 1992 . and Lillard, el at ,1989. and. Lillard, el at , (1990).

The food safety and inspection service (FSIS) of the United States Department of Agriculture (USDA) On July 25.1996, published a final rule on pathogen reduction Hazard Analysis and Critical Point (HACCP)system (PR/HACCP) . The (PR/HACCP) rule requires meat and poultry plants under federal inspection to take the responsibility for , among other things reducing the contamination of meat and poultry products with disease causing (pathogenic) bacteria . Reducing contamination with pathogenic bacteria is a key factor in reducing the 10 number of deaths and illnesses linked to meat and poultry products . The preamble to the final rule describes on overall

system in which preventive and corrective measures . The HACCP system , which is a science based and systemic identifies specific hazards and measures for their control to ensure the safety of food , HACCP is tool to asses hazards establish control systems that focus on prevention rather than relying mainly on end - products testing . HACCP system is capable of accommodating change , such as advance in equipment design , processing Overview of Biological, Chemical and Physical Hazards (Pathogen Reduction/HACCP Regulation, USDA, 1996 (Hazards are not limited to the following information).

Microbial food safety is an increasing public health concern worldwide. It is estimated that each year in the United States there are approximately 76 million food-borne illnesses (MEAD et al., 1999) . Studies worldwide have shown that *Campylobacter*, *Salmonella*, and *E. coli* are often present in fresh meat and poultry (Todd , 1997) . Food contamination with these pathogens can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation. Numerous epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens (Petersen, and James. 1998). Despite the controls that have already been put into place, *Salmonella* infection arising from contaminated food continues to be an immense problem with millions of cases occurring annually throughout the world. In addition to the misery caused, financial loss is enormous.

Detection of *Salmonella* before contaminated foods can be consumed is therefore an essential feature of safeguarding public health and incidentally preserving the reputations and fortunes of food manufacturers and processors(WHO 2003) .

Chapter Two

Materials and Methods

2.1 Study area:

Khartoum State is the largest city and capital of the Sudan, located in the semi-desert area between the latitude 15.08° N to 16.39° N and longitude 31.36° E to 34.25° (Ministry of Animal Resources and Fisheries Informational Center 2015).

The samples were taken from Jabelawlia locality which is located in southern of Khartoum State, and includes many farms and slaughterhouses.

2.2 Type of Samples :-

A total of 70 swab sterile cotton swabs samples were collected during April – June 2017, from two types of poultry slaughterhouses namely; automatic and traditional (manual).

2.3 Steps of slaughtering :

2.3.1 Automatic slaughter house:

Stunning.

Killing and bleeding by cutting the jugular veins.

Collection of blood . travels through a blood collection tunnel.

Scalding. To loosen the feathers, the birds are held in water of temperature 50°C to 60°C.

Defeathering . Feathers are mechanically abraded from the scalded birds.

Washing . The defeathered carcasses were sprayed with cold water .

Opening of the carcass .

Inspection of the viscera.

Evisceration, removal of head , feet and viscera.

Sorting of the viscera to recover heart, liver and gizzard.

washing to remove blood and to loosen tissues.

Chilling of the carcasses with cold water .

packing and weighing .

Chilling and freezing.

2.3.2 Manual slaughter house :

Killing and bleeding by cutting the jugular veins.

Collection of blood.

Scalding. To loosen the feathers, the: birds are held in water of temperatures ranging from 50°C to 60°C.

Defeathering. Feathers are manually abraded from the scalded birds,

Washing.

Evisceration .

Opening of the carcass by cutting manually.

Evisceration, removal of head, feet and viscera.

Inspection of the viscera.

washing to remove blood and to loosen tissues.

Chilling of the carcasses in cold water .

packing and weighting.

Chilling using ice , travelling and freezing .

35 swabs samples from each type in two stages during slaughter processes , samples were also taken from worker's hands were taken , Distribution of samples is shown in table (1) . Swab samples were taken from poultry carcasses first after scalding and secondly after cold water “chilling”, from three sites, namely ; leg, neck and brisket . Samples were taken randomly and transported in icebox to the laboratory .

2.4 Sampling Technique :

Swabs were soaked in buffer saline used for skin scraping (out side) for all samples.

Neck with skin from out surface .

Hand without gloves, from the inside of the hands .Any stage of slaughtering had different workers . Swabs were rubbed over an area of 1cm² . and were placed in 2 ml sterile normal saline.

Table 1:

Swab sample from different sites and steps in slaughter houses

Sample	Automatic (No)	Manual (No)	total
Leg After Scalding(AS)	4	4	8
After Chilling (AC)	4	4	8
Neck After Scalding(AS)	4	4	8
After Chilling (AC)	4	4	8
Brisket After Scalding(AS)	4	4	8
After Chilling (AC)	4	4	8
Workers hand After Scalding(AS)	3	3	6
After Chilling (AC)	3	3	6
After slaughtering (from leg)	5	5	10
total	35	35	70

2.5 Equipment :

Micro-pipette (0.01 ml –one ml) , iron loop , glass loop , bottles , flasks, filter papers , Durham’s tubes , vortex , automatic autoclave , incubator , tips, oven and microscope .

2.6 Sterilization:

Normal Saline, media , tips were sterilized using the autoclave at 121⁰C for 15 minutes . Test tubes and flasks were sterilized using dry heat oven at 160⁰ C for 1 hour .

2.7 Preperation of Culture Media :

2.7.1 Blood Agar (Oxoid DM 100D)

Blood agar was prepared according to Barrow and feltham,(1993). Ten ml sterile defibrinated sheep blood was added to 90 ml nutrient agar which was melted and cooled to 50°C. The blood agar after mixed well was distributed (15-20 ml) under flame into sterile Petri dishes and allowed to solidify at room temperature. The prepared plates were kept in the refrigerator.

2.7.2 MacConkey's Agar (Oxoid CM5)

MacConkeys agar (Oxoid CM5) contained peptone 20 grams, lactose ten grams,bile salts five grams, neutral red 0.075 grams and agar 12 grams. pH was adjusted to 7.4 . Forty grams of the dehydrated medium were suspended in one liter of distilled water dissolved by boiling, then sterilized by autoclaving at 121°C for 15 minutes.

The medium was dispensed in sterile Petri dishes .

2.7.3 Nutrient agar (610036 LioFil chem) .

Nutrient agar was obtained in a dehydrated form the medium contained, Lab-lemco powder one gram, yeast extract two grams, peptone five grams, NaCl five grams, and agar 15 grams per 1000 ml. pH was adjusted to 7.4 . The medium was prepared by adding 28 grams of dehydrated medium to 1000 ml distilled water and dissolved by boiling and sterilized by autoclave at 121⁰ C for 15 minutes under pressure 15 lb per square inch. And then distributed in the plate .

2.8 Special media

2.8.1 liquid media

2.8.1.1 Glucose test (sugar fermentation test) :

The medium was prepared by adding 1% of the sugar to peptone water with Andred's indicator sterilized in Autoclave at 121⁰ C for 15 minutes. Packaging was in bejow vials with Durham's tubes in side , and inoculated the tested bacteria and incubated at 37⁰ C for 7 days when changing the colour from pink to yellow its positive with gas if gas accumulated at upper part of the durham's tube or without gas by acid production only, or no changing in colour its a negative result (Barrow and Feltham,2003)

2.8.2 Semisolid media

2.8.2.1 Oxidation Fermentation (O F) test:-

Test reagent is promothymol blue and the medium is Hugh and Leifson's .Two tubes were inoculated with bacteria one with a paraffin oil and the other tube without oil and incubated at 37⁰ C for two weeks (Hugh and Leifson , 1953) .

result read:

- Positive when change the colour from green to yellow in tube without oil its oxidative .
- Change in colour in two tubes its positive fermentative .
- No change in two tubes its negative ; not (oxidative) or fermentative (negative result) .

2.8.3 Eosin Methylene Blue Agar (Micro Master DMN092)

Eosin Methylene Blue Agar (EMBA) plated on to a selective medium , it was prepared and incubated at 37 ° C for 24 h for isolation and identification of *E. coli* . *E. coli* colonies from samples were identified by greenish metallic sheen by reflected light, and dark purple centers by transmitted light on

EMBA plates, fermentation in *E.coli* and by the presence of bluish fluorescence under a long wave (365 nm) ultraviolet ray (Bridson , 1998)and Jones , *et al*,1991)

2.8.4 Salmonella Shigella Agar (S.S Agar)(Oxoid CM0099) :

Salmonella Shigella Media is a selective medium for isolation of *Salmonella* and *Shigella* species , also to differentiate between Lactose and non-Lactose fermenters . and absorption the bile salt .(MacFaddin1985) . 63g Suspended in 1 liter of distilled water brined to the boil with frequent agitation , and allowed to simmer gently to dissolved the agar cooled to about 50⁰C , mixed , and poured into petri dishes .

2.9 Stainig method

2.9.1 Gram stain ;

In sterile slide using sterile wire loop a drop of normal saline was mixed with part of isolated colony and spread , dried with air and then fixed by flame three times .

Crystal violet was added to cover the slide for 1 minute and washed with distilled water , Lugols Iodine was added for 1 minute and washed using distilled water ,slide decolourization by using ethyl alcohole for10 second and washed immediatly , Sufranin for 30 second and using distilled water for wash then dried with natural air , smear was examined under microscope (x100) oil immersion (Harrigan and McCance ,(1976) .

2.10 Bacterial Count ;

Counting bacterial was performed using the method of Miles and Misra (1938) . a series of test tubes containing 9 ml of normal saline each . 1 ml from original suspension was taken using micropipette to the first test tube shaking and the same procedure was used for the rest of tubes using sterile

micropipette tips every time . a drop from each dilution was cultured in blood agar. Incubated at 37⁰ C for overnight . The numbers of colonies were counted and calculation was done according to the Farmioloae method .

Tube diluted number

2.11 Bacterial culture ;

Samples were cultured directly from swabs on to *Salmonella/ Shigella* media to isolate *Salmonella spp* and on to Mac Conkey's agar . Incubated at 37⁰C overnight . Subcultured on nutrient agar to get pure culture. Incubated at 37⁰C for overnight (Barrow and Felthman 2003).

Smears were made from culture and stained using Gram stain and examined under microscopic oil immersion linse x100 .

2.12 Primary tests :

2.12.1 Catalase test :

Clean microscope slide , afew drops of Hydrogene Peroxide (H₂O₂ %3) and a small part of a bacterial culture were mixed using glass or woody loop when bubbles arise the test was considered positive .

2.12.2 Oxidase test :

Afilter paper was soaked in the of oxidase test reagent (Tetramethyle-p-phenylene Di-amine Dihydrochloride) diluted with sterile distilled water . A small amount of an overnight culture, on nutrient agar , was picked with sterile glass loop and rubbed on the filter paper surface placed in a Peri dish , positive result give violet colour (Barrow and Feltham ,2003) .

2.13 Analytical Profile Index (API20E) ; (API20E(2012))

Identification of isolated bacteria were performed using API20E (Analytical Profile Index) for Enterobacteriaceae that were oxidase positive. The API20E strip consisted of 20 microtubes as indicated in API strip some wells were covered with oil. These tests were inoculated with a bacterial suspension that reconstitutes the media. During incubation, metabolism produced color changes that were either spontaneous or revealed by the addition of reagents. The reactions were read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index or using the identification software. (API20E Manual 2012)

Chapter Three

Results

3.1.Total Viable Bacterial count :

3.1.1 Bacterial load in automatic slaughterhouse :

In the 70 swab samples examined bacterial load in the automatic slaughter house ,The total bacterial count in the automatic slaughter house in the scalding stage was highest at the worker's hands (120 cfu/ cm²) , leg (99 cfu/ cm²), brisket(57 cfu / cm²) and the neck (4 cfu / cm²). (table2) .

For chilling stage the highest count was in the legs (60 cfu /cm²) , neck (57 cfu/ cm²) , brisket (3 cfu /cm²) and worker's hand (3cfu /cm²). The highest bacterial count found in this type of slaughtering was in the worker's hands during scalding stage (120 cfu/ cm²) and the lowest was in the brisket and worker's hands during chilling stage (3 cfu/ cm²) . (table 2)

Table 2 :

Mean and standard deviation (SD) of total viable bacteria counts(fcu/ cm²) in different points in broiler carcasses in the automatic slaughter house .

Stage \ Site	Leg (n= 13)	Neck (n=8)	Brisket (n= 8)	Worker'shand (n =6)
Afterslaughter	2.6 (±5.9)	not done	not done	not done
Scalding	9.9 (± 9.9)	0.4 (± 2)	5.7 (± 7.5)	12.0 (±10.9)
Chilling	6.0 (±7.7)	5.7 (±7.5)	0.3 (± 1.7)	0.3 (±1.7)

3.1.2 Bacterial load in manual slaughters houses :-

The highest bacterial load in manual slaughtering at the scalding stage in the legs (205 cfu/cm²) , neck (181cfu/ cm²), brisket (170 cfu/cm²) and worker's hand (112cfu/cm²) . In chilling stage the worker's hands recorded(125cfu /cm²), leg (105 cfu cm²), brisket (89 cfu/cm²) , neck (50 cfu/cm²)and after slaughtering (171cfu/cm²).The highest one in the leg in scalding (205cfu /cm²) , the low bacterial load in the neck (50 cfu /cm²)in chilling stage (table 3).

Table(3) :-

Mean and standard deviation (SD) of total viable bacterial counts log 10 cfu/Cm² in different site of broiler carcasses in the manual slaughterhouse .

Stage \ Site	Leg (n= 13)	Neck (n= 8)	brisket (n= 8)	Worker'sHand (n= 6)
Afterslaughter	17.1(± 13.07)	not done	not done	not done
Scalding	20.5 (± 14.3)	18.1 (±13.4)	17.0(± 13.03)	11.2 (± 10.5)
Chilling	10.5 (± 10.2)	5.0 (± 7.07)	8.9 (±9.43)	12.5 (± 11.1)

As show in table (4) manual slaughtering mean and standard deviation in diferrent stages was 17.1(±13) in after slaughter stage, scalding 18.5(±13,6) , chilling 8.1(±9), worker's hand 11.8(±10.8) cfu/cm². Automatic slaughter mean and standard deviation was in after slaughtering stage 2.6 (± 5), scalding 5.3(±7.2) , chilling 4.0 (± 6.3) , Worker's hand 0.6 (± 2.4)cfu/cm² (table 4).

Table(4):

Mean and standard deviation (SD) of total viable counts of bacteria in different point in slaughter houses.

Points	Mean log ₁₀ cfu /cm ³		Significant different
	Standard deviation		
	Automatic slaughtering	Manual slaughtering	
After slaughtering	2.6 (± 5)	17.1 (±13)	*N.S
Scalding	5.3 (± 7.2)	18.5 (±13.6)	*N.S
Chilling	4.0 (± 6.3)	8.1 (± 9)	*N.S
Worker's hands	0.6 (± 2.4)	11.8 (± 10.8)	*N.S

There was no significant differences between these operational points, N.S.

*Non significant : (P > 0.05).

3.3 Bacterial isolation and identification :

Isolation and identification of *E.coli* in two types of slaughter house in different sites in broiler carcasses is shown in table (5).

3.3.1 API20E Test :-

Table(5) :-

Results of API20E test for identification of *E.coli* isolates .

	Automatic			Manual							
	1	2	3	1	2	3	4	5	6	7	8
NPG	+	+	+	+	+	+	+	+	+	+	+
ADH	-	+	-	+	+	-	+	-	-	+	-
ODC	+	+	-	+	+	-	+	-	-	+	-
CIT	-	-	-	-	-	-	-	-	-	-	-
H2S	-	-	-	-	-	-	-	-	-	-	-
URE	-	-	-	-	-	-	-	-	-	-	-
TDA	-	-	-	-	-	-	-	-	-	-	-
IND	+	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-	-
GEL	-	-	-	-	-	-	-	-	-	-	-
GLU	+	+	+	+	+	+	+	+	+	+	+
MAN	+	+	+	+	+	+	+	+	+	+	+
INO	-	-	-	-	-	-	-	-	-	-	-
SOR	+	+	-	+	+	-	+	-	-	+	-
RHA	+	+	+	+	+	+	+	+	+	+	+
SAC	+	+	+	+	+	+	+	+	+	+	+
MEL	+	+	+	+	+	+	+	+	+	+	+
AMX	-	-	-	-	-	-	-	-	-	-	-
ARA	+	+	+	+	+	+	+	+	+	+	+

E.coli was found in 8(22.85%) samples in manual slaughtering house , 4 samples from scalding stage (11.42%) from a total numbers of samples in scalding stage . 2 (5.71%) samples from chilling stage , 2 (5.71%) samples from after slaughtering stage . Table (6)

Table (6) :

Frequency of *E.coli* isolation in different stages in manual slaughtering samples

	Samples number	percentage	Positive samples	Percentage
Valid				
After slaughtering	5	14.3%	2	5.71%
Scalding	15	42.9 %	4	11.42%
Chilling	15	42.9%	2	5.71%
Total	35	100.0 %	8	22.85%

E.coli was found in 3 (8.57%) in automatic slaughtering house samples from after slaughtering stage . (table 7)

Table 7 :

Frequency of *E.coli* isolates in different sites of automatic slaughtering samples

	Frequency	Percentage	Positive samples	Percentage
Valid				
leg	8	22.9 %	0	0
Neck	8	22.9 %	0	0
brisket	8	22.9 %	0	0
Hands	6	17.1 %	0	0
Afterslaughter(leg)	5	14.3 %	3	85.7%
Total	35	100.0 %	3	

3.4 Bacteria in chicken samples from stages and sites :

Chicken samples were collected from three stages these were after slaughter 10(14%) , chilling and scalding 30 (42.9 %) from each type of slaughter house .

The chicken samples and swabs from worker's hands collected from 60(85.7%) in chilling and scalding stages in two types of slaughtering and 10(14.3%) from afterslaughter , only 5(7.%) samples were positive for *E.coli* from the afterslaughter stage.

While all samples from chicken parts and worker's hands were negative for *Salmonella spp* (table 8) .

Table 8:

Distribution of *E.coli* and *Salmonella spp* by using API20E test

Variables	Frequency	Percentage	Total
<i>E .coli</i> presence			
Negative sample	59	85.7%	
Positive sample	11	14.3%	70
<i>Salmonella</i> presence			
Negative sample	70	100%	
Positive sample	0	0.0%	70

From a total of 12 samples of worker's hands only 2 (16.66%) were positive for *E.coli* while 10(83.34%) were negative .On the other hand chicken parts samples showwd a pit higher presence of 9(18%) positive and 49(82%) negative for *E.coli* (table 9).

Table 9 :

Occurance of *E.coli* in worker's hands in comparison with chicken parts

Distribution	Frequency	Percentage	Total
<i>E .coli</i> presence			
1-Hand swap			
Negative sample	10	80%	12 sample
Positive sample	2	20%	
2- Chicken parts			
Negative sample	49	82%	58 sample
Positive sample	9	18%	

In the automatic slaughtering type , out of 11 samples only 3(27.3%) samples were found positive for *E.coli* by using the API20Etest as follow. two 2(18.2%) at the after slaughtering point and 1(9.1%) at the scalding point (table 10).

Table 10:-

Identification of *E.coli* in automatic slaughtering by using API20E test (N=1)

Point	<i>E.coli</i>
Afterslaughter	2 (18.2 %)
Scalding	1 (9.1%)
Chilling	-
Hand	-
Total	3 (27.3 %)

Manual slaughtering type result showed , eight (8) samples were found positive for *E.coli* by the API20E test as follows: 2 sample (5.71 %) . in after slaughtering stage, 2 sample (5.71%) at scalding stage, 2 sample (5.71%) from the neck area at chilling stage and 2 sample (5.71%) from the leg and brisket area and worker' hands during scalding (table 11) .

Table 11 :-

Identification of *E.coli* in manual slaughtering at different stages from different sites .

Stages	<i>E.coli</i>
After slaughter	2 (5.71%)
Scalding	2 (5.71 %)
Chilling	2 (5.71%)
Hands	2 (5.71 %)
Total	8 (22.85 %)

Chapter Four

DISCUSSION

The level of *Enterobacteriaceae* in poultry carcasses can be routinely used as an indicator of improper hygiene methods during processing and incorrect storage conditions, which can lead to the proliferation of pathogens, such as *Salmonella*, and toxin production (Roberts *et al.*, 1995).

Bacterial load (total viable count-TVC) from the broiler carcasses in automatic slaughterhouse in this study was found to be high in the chilling stage and this is in agreement with Irena Svobodova *,et al*, (2011), who stated that microbial contamination of poultry carcasses can evaluate the impact of four processing steps (plucking , evisceration ,washing and chilling) during one year in Czech slaughterhouse , where TVC of *E.coli* decreased during processing from 4.6 log cfu /cm² with major impact of washing and chilling on *E.coli* TVC Gořksoy, *etal*, (2004).

In this study bacterial load is high in chilling , *E.coli* was also high 10 (14.2%) ,It is obvious that the variability in microbial counts (especially after washing) indicated the need for use of prerequisite programs). The increase of the microbial contamination at chilling stage in this study, disagree with G Gabeer *et al* (2012) .

In a majority of the samples had coliform bacteria specially after de-feathering (Ahmed . *et al* 2013), this result disagree with the present finding .

In this study *E.coli* in broiler carcasses was found lower in automatic slaughterhouse with 3(4.2%) , than *E.coli* in broiler Chicken carcasses in Republic of Trinidad and Tobago (38.7%) by A. Thomas, *et al* (2006) , *E.coli* was found to be the most contaminant of broiler meat , eggs , and products in Khartoum State (34,6%) by Egbal Munir *et al* 2011) .

In this study *E.coli* in broiler carcasses was found in automatic slaughterhouse a three samples 3(4.2%) , its comparable to the study performed in Egypt where *E.coli* represented (4%) in chicken carcasses (Hemmat ,(2015) and 2% (Cardoso ,*et al* , 2006) Guven ,*et al.*,(2002) found the *E. coli* as 0% in 1 g of sample from processed raw goose carcasses marketed in Kars (Turkey) this results disagree with the present finding .

In this study *Salmonella spp* was not found in all samples this disagrees with the study to isolate and identify *Salmonella spp* from chicken slaughterer under different processing condition , supermarket , and chicken slaughtered in modern processing units in karnakata , India , tested breast and thigh muscle *Salmonella* was high in thigh meat (31.99%) , compared to breast muscle (24.88 %) (Wilfred *et.al.* 2010) .This study does not agree with the study which detected *Salmonella spp* in broiler carcasses in Washington State University in (1999 and 2000) .11.99% of the samples in which *Salmonella spp* was isolated from poultry products , poultry and poultry environment (Parimal *et al* 2001) .Microbiological quality and contamination of chicken meat is of great importance . The finding of *Salmonella spp* was (10.60%) by lidija koza *et al*(2006) .In greater Washington D.C area ; *Salmonella* was isolated in 9(4.2%) Out of the 520 samples which were examined by bacteriological tests, 45 samples (8.65%) had positive results for *Salmonella* as follows: 29 of 400 samples (7.25%) from chickens, 14 of 90 samples (15.55%), from pigeons, and 2 of 30 samples (6.66) from ostriches were positive for *Salmonella* strains by using serological test (.Akbarmehr (2010) , In this study no *Salmonella spp* had been isolated from the different part of poultry in Khartoum State JabalAwlia locality .

CONCLUSION.

- 1- Total Viable Count of bacteria is high in manual slaughtering than automatic slaughtering systems .
- 2- Highest bacterial count found in leg during scalding in manual slaughtering
- 3- Lowest bacterial count in hands and birsket , after chilling in automatic slaughterhouse .
- 4- Contamination can happen after washing with cold water .
- 5- Bacterial contamination of broiler carcasses can occur in the two types of slaughter houses namely automatic and manual ones .

RECOMMENDATION

- 1- Good personal hygiene measures are required .
- 2- Hygiene practice measure in slaughter houses are needed .
- 3- Awireness between slaughterhouse workers might help reducing meat contamination .

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