

## INTRODUCTION

Poultry industries in Sudan began in 1926 by enter a group of Yandotte Chicken from British ,followed of central poultry farm in Khartoum Bahri in 1951 . in Sudan broiler chicken population was estimated to be 22.5 million chicks . Traditional sector ( small farms) produced about 60% of the total broiler production and modern sector (companies) produced the rest . According to field survey in 2009 the production of broilers was 17.5 million chick , and the poultry factories in Khartoum State , were about 10 factories of poultry broilers production with capacity of 25000 tons /hour , Nagla (1998) .

poultry meat production in Sudan in 2014 about 90 million tons per year . poultry meat production in 2005- (0.75)kg/capita and 1kg/capita in 2015.Khartoum State produce 90% of Sudan production. Mohamed ( 2014).

Most countries have been worried about food-borne diseases nearly in developing countries due to food problems reported cases economic and social costs effect around the world Zhao *et al.*, (2001) Poultry meat can simply contaminated with microorganisms, thus modern processing needs an elevated rate of through put to meat consumers demand Kabour (2011) However ,many healthy broilers entering poultry processing plants might be highly contaminated with microorganisms and serve as healthy carriers of these microorganisms. They might be carrying food-borne pathogens or spoilage microorganisms such as *Salmonella species*, *Campylobacter species*, *Clostridium per fringes*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aurous* and other bacteria (Mead et al.,1994).

Poultry meat is essential part of animal food market and its production is increasing to satisfy the public demand world-wide as relatively in expensive cost if compared with other animal protein sources (Bryan 1980., Bok et al.,1986. ,Anand et al. , 1989).

The microorganisms in different part of carcass ,carried out on food from origin animal, 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).

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, the series of microorganisms on the surface of carcasses which 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 over traditional methods, and results obtained in study from eight slaughter houses suggested that HACCP systems can maintain or even improved food safety(Cates et al., 2001).

During processing of poultry carcasses microbial contamination inevitably occur as a consequence of processing procedures employed, at each stage processes opportunity exists for contamination of carcasses by microorganisms from the processing plant by cross contamination from the birds , numbers of bacteria or carcass surfaces vary considerably by different stage of processing (Barnes ,1960. Lahellec et al., 1972).

The contamination and or cross - contamination of carcasses, during slaughter process were demonstrated and results indicated presence of bacteria potential public health significances (Doyle, 1991, Biss & Hathaway 1995). Also dirty work hands ,clothes, equipments of slaughterhouse . acts as intermediated sources of contamination of meat (Gill, 1998; Gilmour et al., 2004).

Also due defearthening 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). Quality of poultry meat during slaughtering and packing and hygienic statute of slaughterhouse(Lillard 1990). Monitoring of all steps of process aiming the food safety of final product HACCP in poultry industry is extremely important it involve the constant, this safety program to serve both internal and external market (Jimenez et al., 2002, Mead 2004 , Galhardo et al., 2006).

Each year millions of people worldwide suffer from food borne diseases (WHO,2000)and illness resulting from the consumption of contaminated food had become one of the most worldwide public health problems is contemporary society (Notermans et al., 1995).

Some microorganisms such as *Salmonella spp.*; *Escherichia coli* 0.157 and *Listeria Monocytogenes* pose a threat to consumer health (Gustavasson and Borch 1993,Samelis et al., 2001) .During processing of poultry carcass microbial contamination inevitably occur as a consequence of processing produces employed , at each stage of process, opportunity exists for contamination from other birds , numbers of bacteria on carcass surfaces vary considerably by different stage of processing (Barnes ,1960 ; Lahellec et al., 1972 , the contamination and/

or cross contamination of carcasses , during slaughtering process were demonstrated and results indicated presence of bacteria of potential health significance (Doyle 1991 ,Biss and Hathaway 1995.) Also , dirty workers hands , clothes , equipments of slaughterhouse acted as intermediated sources of contamination of meat (Gill ,1998 , Gilmour et al., 2004 , Abdelsadig 2006 ,Abdalla et al., 2009).

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).

HACCP principles for control of the microbiological quality of intended foods to humans consumption (FAO 2012). 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).

**Objectives:-**

- 1- To indentify microbial contamination point for establishing the critical control points( CCP) in poultry automatic slaughter house
- 2- To evaluate the bacteriological level contamination of poultry meat with *Salmonella spp. and Escherichia coli* during slaughtering process .

## **Chapter One**

### **literature Review**

#### **1.1 History of HACCP : -**

HACCP has become synonymous with food .

HACCP system, which is science based and systematic , identified specific hazards and measures for their control to ensure the safety of food .Roberts,(2001)

HACCP is tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end - product testing and inspection.

HACCP :A system which indentifies , evaluates , and control hazards which significant for food safety .Ying Zhen (2011).

#### **1.2.Food Safety :**

Food safety is any plan to keep food safe and to decrease hazard of food borne cases or any plants causing public health .

Food Safety related to Hazards

Hazard Analysis Critical Control Point (HACCP) is a systematic, scientific approach to 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 can include biological (pathological and microbiological for beef slaughter), chemical or physical contamination of food products.

The United States Department of Agriculture (USDA) published a final rule in July 1996 mandating that HACCP be implemented as the system of process control in all USDA inspected meat and poultry plants. As part of its effort to assist establishments in the preparation of plant-specific HACCP plans, FSIS determined that a generic model for each process defined in the regulation will be made available for use by the industry.

In May 1996, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) awarded Contract Number 53-3A94-6-04.

### **1.3. Poultry Model**

#### **1.3.1. The International Meat and Poultry HACCP Alliance for the development of ten generic HACCP models.**

1. Not Heat Treated, Shelf-Stable (dried products, those controlled by water activity, pH, freeze dried, dehydrated, etc.)
2. Heat Treated, Shelf-Stable (rendered products, lard, etc.)
3. Heat Treated Not Fully Cooked, Not Shelf-Stable (ready to cook poultry, cold smoked and products smoked for trichinae, partially cooked battered, breaded, char-marked, batter set, and low temperature rendered products, etc.)
4. Products with Secondary Inhibitors, Not Shelf-Stable (products that are fermented, dried, salted, brine treated, etc., but are not shelf-stable)
5. Irradiation (includes all forms of approved irradiation procedures for poultry and pork)

6. Fully Cooked, Not Shelf Stable (products which have received a lethal kill step through a heating process, but must be kept refrigerated. This includes products such as fully cooked hams, cooked beef, roast beef, etc.).

7. Beef Slaughter

8. Pork Slaughter

9. Poultry Slaughter

10. Raw Products - not ground (all raw products which are not ground in their final form. This includes beef trimmings, tenderized cuts, steaks, roasts, chops, poultry parts, etc.

All plants shall have Sanitation Standard Operating Procedures (SSOPs). Good Manufacturing Practices (GMPs) and Standard Operating Procedures (SOPs) may be in place as the foundation of the HACCP program. Good Manufacturing Practices are minimum sanitary and processing requirements applicable to all companies processing food. Standard Operating Procedures (SOPs) are step-by-step directions for completing important plant procedures. SOPs should specifically describe the method for conducting and controlling the procedure. SOPs should be evaluated regularly (i.e., daily) to confirm proper and consistent application, and modified as necessary to ensure control.

Each generic model can be used as a starting point for the development of your plant-specific plan reflecting your plant environment and the specific processes conducted. The generic model is not intended to be used..

### **1.3.2. Specifics about this Generic Model**

1/ Products Included In This Model: This model deals only with poultry slaughter. The product samples include broilers and turkeys.

2/ Items Addressed:

This model does not address certain aspects of product safety, such as Sanitation Standard Operating Procedures (SSOPs). Good Manufacturing Practices (GMPs) and Standard Operating Procedures (SOPs) may be in place as the foundation of HACCP. Critical Control Points.

3/ The Critical Control Points: in this model were established by the team members of the workshop. Some products or processes may require fewer or more CCPs depending on the individual operation.

4/ Product Flow: In the product flow, the general processes were included; however, order of flow varies. The product flow of every HACCP plan should be specific and accurately reflect the processes involved at each plant.

5/ Safety vs. Quality: Several parameters have been discussed to ensure a safe product. Only parameters relating to product safety were discussed. Quality issues were not addressed in this model.

6/ Critical limits selected: must be based on the best information available to provide a safe product and yet be realistic and attainable.

Processors must keep in mind that any product which does not meet a critical limit must have a Corrective Action taken on the product before being released from the plant.

Process Authority. Reference may have been made about a "Process Authority" in this model.



7/ A Process Authority: may be an in-plant employee who has had specialized training, an outside consultant, or other professional.

8/ Record-keeping: Record-keeping is an important part of the HACCP plan. Lack of accurate, current records may be cause for withholding or suspending inspection from a plant.

9/ Chain of Custody: Chain of custody refers to the point at which a plant gains control of the meat. This is particularly important to know the history of incoming meat products.

Requiring a HACCP plan from the supplier will in effect, extend the chain of custody to the supplier.

10/ Sampling Procedures: Each plant must establish a sampling plan to verify critical control points (biological, chemical and physical) in the operation.

The procedures will be based on prior knowledge about the problem areas and not necessarily on random testing. A Process Authority may help establish these sampling procedures which are most likely to identify a problem if it exists.

#### **1.4. Conception of HACCP System : -**

On July 25.1996, the food safety and inspection service (FSIS) of the United States Department of Agriculture (USDA) 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

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).

### **1.5.Chic ken Processing**

Unloading

Hanging

Stunning

Killing

Bleeding

Scalding/Washing

Picking

Poultry Slaughter Model

Washing

Head Removal

Hock Cutter

Transfer/Rehang  
Oil Gland Removal  
Neck Breaking  
Venting  
Chicken Processing Cont.  
Opening  
Evisceration  
Presentation  
Inspection  
Trimming  
Liver/Heart Harvest  
Visceral Removal  
Poultry Slaughter Model  
Gizzard Harvest  
Lung Removal  
Liver/Heart Chill  
Reprocessing  
Salvage  
Salvage Chill  
To Storage or Shipping

Cut Neck

Vacuum

Wash

Inspect-QA/USDA

Chill

Giblet Pack

Gizzard Peel

Gizzard Chill

Giblet Pack

Crop Removal

Neck Removal/ Harvest

### **1.6.Poultry Slaughtering house Processing : -**

The field of poultry processing involves converting live poultry into food products for human consumption . The main concerns in poultry products are safety and quality characteristics (Sams, 1994 ) .

Hinton et al., (2004). Reported cross-contamination in all processing steps and even successive days the same bacteria could be found. Government control action mainly focuses on the control in processing with especial attention given to water chilling and reprocessing of carcasses. Carcasses decontamination is an important step here HACCP in poultry processing is gaining attention , unfortunately HACCP in poultry processing alone is not enough to maintain this

downward tendency . previously there is a clear need to control pathogens in live birds that are ready for slaughter.

#### **1.6.1.Slaughtering Stunning and Killing :**

After the birds have been transferred to the moving shackles , they are usually stunned by running products unconsciousness , but it does not kill the birds are killed either by hand or by a mechanical rotary knife that cuts jugular veins and carotid arteries at the neck. Any birds are not killed by the machine are quickly killed by person with is knife assigned to the bleed area . The birds are permitted to bleed for fixed amount of time ,depending on size and species . Any birds that is not properly bled will be condemned (Encyclopedia Britannica , 1998 ).

Hafez (1999) mention that birds arriving for slaughter are heavily contaminated with microorganisms which are carried in the intestine , on the skin and among the feathers . In the Modern fully automated poultry processing plant it has become impossible to isolate individual carcasses from others material or from equipments , employees and other material essential for production. consequently , subsequent cross-contamination can occur at all stages of process Automated unloading of containers followed by transportation of live birds into hanging area , whether via-gas-stunning tunnel or not. poses microbiological challenges when cleaning these complex and systems .

#### **1.6.2.Scalding ,Plucking and Evisceration :**

Stals (1996). Reported that most critical points, for cross-contamination during processing are scalding , plucking , evisceration. At the scalding stage many bacteria are washed from carcasses and the result in contaminated scald water . Treatment of scald water with acid (acetic or lactic acid ) is further application that could be used to reduce

the number of microorganisms . The use of high pressures (800 bar) for the scald water reduce the number of Enterobacteriaceae (Van and Mudder 1996).

Kim et al.,(1993). Reported no different in microbial CFU Counts between scalding temperature of 52c° , 56c° and 60c°. Change in PH of scald water can reduce D-values of microorganisms in scald water (Humphrey et al., 1984 , Bolder 1998). High scalding with result in loss of birds epidermis and causes difference of growth condition for micro flora, especially in case of water chilled non- frozen storage , low scalding and dry chilling leads to dry skin with different attachment and growth for markets (Hinton 2000). Pluckers can be turned out during cleaning allowing more efficient hygiene practices , plucker fingers become contaminated , washing pluckers during and after use is not only important to remove feathers , but also delay bacteria from attaching to the processed carcasses . Regular rising of carcasses with help to inhibit attachment of microbe ; especially those that exhibit freshly produce fecal material during plucking process , The close contact of plucked fingers with carcasses may Ruben organic and microorganisms . Application of water in the pluckers can considered as processing aid, as feathers are easier removed from the pluckers , and water provides lubrication . Air in the plucking area can be highly contaminated (Berrang et al., 2004).

Evisceration is removal of viscera by using series of interconnect machines , reducing fecal material at slaughter is an important practices which reduce contamination during processing which can occur because of leakage of contents of intestinal tracts on to the carcasses or through spoilage resulting from ruptures of gastero-intestinal tracts and viscera during evisceration (Russell,2001. Mead 2004 ).

Castaned et al., (2005). Found that evisceration of carcasses can result in severe cross-contamination from feces , mainly as result of damage to the intestine as well as contact between intestine and carcasses.

It is benefit to wash the carcasses at different stages of processing after defeathering and not just before chilling . the use of inside - outside carcasses washers remove visible fecal contamination but does not eliminate those bacteria that have become attached to carcasses surface (Mead et al. 1997).

Bolder ,(2007). Mentioned that removal of an intact intestinal package is very important to prevent the spread of fecal material and bacteria on the carcasses. Final washing and inspection of carcasses is now fully automated , so human quality checks are seldom necessary at the end of evisceration line . Damage and soiled carcasses can be used pre-selection before Vet. checks .

Following bleeding the birds go through scalding tanks . These tanks contain hot water that softens the skin so that the feathers can be removed . The temperature of water is carefully controlled. If retention of the yellow skin color is desired , a soft - scald is used ( about 50c<sup>°</sup> or 122F<sup>°</sup>).If a white bird is desired, a higher scald temperature is used, resulting in the removal of the yellow pellicle. Turkeys and spent hens (eggs - laying birds that have finished their laying cycles ) are generally run at higher temperature --59C<sup>°</sup> to 60C<sup>°</sup> (138<sup>°</sup> to 140<sup>°</sup> F )( Encyclopedia Britannica , 1998).

### **1.6.3.Defeathering : -**

The carcasses then go through the feather - picking machines , which are equipped with rubber fingers specifically designed to beat off the feathers . The carcasses are moved through a sequence of machines .

each optimized for removing different sets of feathers . At this point the carcasses are usually singed by passing through the flame that burns off any remaining feathers ( Encyclopedia Britannica , 1998).

Defeathering or picking achieved by passing the birds through rows of rotating rubber fingers that remove the feathers & help squeeze the remaining blood. mechanical pickers & other items used for processing must be constructed to ensure clean lines (Houston 1985 ). It represents another chance for cross - contamination , consider microorganism like Salmonella have been shown to attach firmly to poultry skin and rubber fingers act as transmitters for contamination . The extent of cross - contamination during plucking is governed by the hygiene of scalding process. Alternatives have been developed including simultaneous scalding & plucking & steam scalding . These minimize cross - contamination with Salmonella spp.

Salmonella are more frequently isolated from carcasses after defeathering than following any other processing operation ( McBride et al., 1980). Following hot or hard scalding , defeathering damages & removes the epidermal layer & exposes a new surface layer .

Contamination either during primary production (e.g. slaughtering ) or further processing & handling ( cross - contamination during processing human to food contamination via food handlers ) . *Escherichia coli* has been isolated world-wide from at (Contamination of poultry properly due to increased used antimicrobials (Miranda et al, men et al. , 2008 ; Adesiji et al., 2011 ). Also due 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).



On study of the affect of processing procedures and overall environmental and hygienic condition of the microbiological quality and safety found heavily contamination at scalding and defeathering with *Salmonella and Escherichia coli* (Abu-Ruwida et al., 1994 ).

The presence of *Escherichia coli* in fresh meat can be attributed to carcass contamination with the gastrointestinal content during processing .The contamination levels recorded in the point of washing in all sites carcasses may be due to unclean management during the washing , (Ali 2007) .

The Mean TVCs obtained from chicken carcasses in following CCPs ; after defeathering, after evisceration , after spray water and hands of employees are similar to that obtained by Kabour (2011). Furthermore , the Mean TVCs are confirming the reports of Mohamed Noor et al. (2012) . who found the same TVCs of some CCPs including : after defeathering , after evisceration , after chilling and employees hands at the same time.

#### **1.6.4.Chilling Storage . Packaging :**

Different types of chilling processes are used all methods may lead to cross-contamination of carcasses but the problem is greater in systems that use water. the risk of cross-contamination can be reduced by the use of chlorinated water and tri sodium phosphate used dip immediately after chilling or before air-chilling reduces contamination with *Salmonella m campylobacter and Escherichia Coli* (Hinton and Corry 1998). the use of low voltage electrical current with low concentration of salt in the chill water has been shown to eliminate *Salmonella Tryphimurium* and

Campylobacter Jejuni from chiller water and reduce the contamination on chickens skin (Li and Slavik 1996).

Chilling with air is becoming more popular worldwide although studies on the bacteriology of air chilling do not show any reducing in pathogens or bacterial counts (Allen et al., 2000 ; Flucky .et al., ).

During immersion chilling in water equilibration of contamination occurs , not only by spread of pathogens from contaminated to uncontaminated carcasses but also in increased uniformity of CFU. counts after the chiller , in comparison with air-chilled carcasses (Smith. et al., 2005).

**Water Chilling : -**

Water Chilling is used a per-chilling step in which countercurrent flow of cold water is used to lower the temperature of carcasses , The carcasses are then moved into a chiller a large tank specific designed to move the carcasses through in specific amount of time . two tanks are used to minimize cross-contamination . Although this renders the chilling process very water-intensive ,it helps to minimize bacterial cross-contamination by diluting the microorganisms washed off the carcasses. thereby preventing recontamination . Water chilling leads to an increase in poultry weight , and the amount of water gained is carefully regulated( Encyclopedia Britannica , 1998).

**1.7. Bacteriology of poultry meat : -**

Microbiological status of broiler carcasses depends on several factors . such as : infection level of living birds and cross contamination during processing (Abu-Ruwaida et al. ,2004 ).

1. The procedure for converting a live, healthy bird into a safe and wholesome poultry product provided many opportunities for microorganisms to colonize on the surface of the carcasses (Bryan , 1980 ; Thomas and McMeekin, 1980 ; Roberts , 1982 ; Grau , 1986 ; Bailery et al ., 1987 ; Connor et al., 1987 ; Banwart , 1989 ; Mead , 1989 ). The significance of bacterial Microbiological Safety and Quality of Poultry Meat.
2. The microbiological safety and quality of poultry meat important to producers ,retailers and consumers ,and both involve microbial contaminants on the processed product .
3. Meat hygiene and safety. It is generally assumed that preventing visible contamination or removing visible contamination from carcasses will enhanced the microbiological safety of meat . They have a potential impact on the food safety or bacteriological quality of poultry carcasses. Heemskerk (2005 ) reviewed the recent literature on the slaughter process and came to the conclusion that improvements on the hygienic situation could only be obtained by intervention at several place in slaughterhouse process at same time .

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 micro-organisms They also added that the factors which influenced the growth of microorganisms and hence the kind of spoilage were the kind and amount of contamination with micro-organisms and its

spread the physical properties of the meat i.e the amount of surfaces exposed to air the chemical properties of meat which included moisture content and pH the availability of oxygen and temperature (Frazier and Westhoff , 1978 )

Drewnaik et al ., (1984) found that there was 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 water decreases the bacteria present on the skin of poultry .

most bacteria found poultry surfaces were found to consist of flora that were present prior to slaughtering and they were picked up during defeathering , pinning from workers hands and knives , from eviscerating or cooling due contamination (Nickerson and Sinkey, 1974).

The sources of contamination in poultry processing plant (Frazier and Westhoff,1978) were two types: exogenous and endogenous.The exogenous contamination of skin and the lining of body cavities occurred during washing , plucking and evisceration , Microorganisms present in food were described by Banwart (1981). They included those acquired during handling and processing and those surviving a preservation treatment and storage Water activity as a factor could play a role in spoilage . Jay (1986 ) pointed out that it might be assumed that all microorganisms existed in water might be existed in foods The hazard of air borne contamination of foods was indicated by Gregry (1961). He claimed that pathogenic organisms might spread in air by foods handlers during sneezing or coughing and deposit on meat surfaces .The micro flora on their hands and outer garments generally reflected the environment and habits of the individuals . The microflora consisted of

genera found on any object handled by the individuals in the addition to those from water, dust and soil.

A related factor to the contamination was the way of holding of feet and heads of fowl on the slaughter line (Jay, 1986) Bryan (1987). However Jay (1986) considered that food handlers to be important source of contamination. In their microbiological survey, (Schuler and Badenhop (1972) found that packing materials might also represent an important source of contamination. Jay (1986) mentioned that the environment of poultry slaughter-house contaminated several genera of bacteria more than in soil, water or other places. Among these were Bactericides, *Escherichia*, *proteus*, *Salmonella*, *Shigella*, *Staphylococcus*.

### **1.8. Microbiological Quality of poultry Carcasses during slaughter house processing :-**

During the slaughter of poultry birds there can be feed contamination of carcasses from gut of the birds which mean bacteria present in spilled gut content is passed on as contaminants of importance is coli form especially *Escherichia coli* and *Salmonella*. *Colibacillosis*, *Salmonellosis*. have been described as the leading causes of food-borne illnesses worldwide (Panisello et al, 2000). Therefore, it becomes important that ensuring consumer health concerns the greater involvement of the sector. *Salmonella* is of an increasing public health concern because they are the most incriminated pathogenic microorganisms of bacterial food poisoning especially present in poultry meat, with infection being through the handling of raw poultry carcasses and products, together with consumption of under cooked poultry meat. (Panisello et al., 2000).

## **1.9. Bacterial Pathogens Associated with Poultry Meat :**

Potential biological hazards in meat poultry include bacteria , toxins ,viruses , protozoa and parasites. Of the microbiological hazards ,the most important are bacteria. Bacteria cause a large proportion (approximately 90% ) of all food borne illnesses. Bacteria that cause human illness , including disease, are termed pathogenic .*Listeria monocytogenes* also is widespread in the environment and is often present in soil. water , and silage. Although *Escherichia coli* also is found in livestock and poultry , most forms of *Escherichia coli* are not pathogenic. *Escherichia coli* O157:H7 is pathogenic. Pathogens associated with poultry are *Salmonella*, *Staphylococcus aureus* ,*Clostridium perfringens* and *Escherichia coli*. *Listeria monocytogenes* and *Campylobacter jejuni* have also been isolated form poultry. Spoilage bacteria most frequently associated with poultry *Pseudomonas spp.*, *Acinetobacter spp.*, *Moraxella spp.*, *Alteromonas Putrefaciens*, *Aeromonas spp.*, *Corynebacterium spp.*, *Flavobacterium spp.*, *Micrococcus* and numerous Enterbacteriaceae.andTompkins.,1984;Evans, 1986;Gill,1986 ; Grau, 1986; Silliker and poultry is vehicle of food borne illness (Bryan, 1980; Todd,1980;Smeltzer,1981;Brown and Baird-Parker.,1982;Mead,1982,Roberts,1982;Ralph andTompkins.,1984;Evans, 1986;Gill,1986 ; Grau, 1986; Silliker and Galois., (1986);Cunningham (1987);Banwart, (1989); Mead, (1989);Zottola and Smith., (1990); Jones et al.,1991.

All of these pathogens have been implicated in widely publicized food borne disease outbreaks associated with the consumption of meat and poultry products

### **1.9.1. Salmonella species : -**

*Salmonella* are main cause of food poisoning from poultry meat (Dougherty , 1974 ; Todd , 1980 ). *Salmonella* are often pathogenic for humans and animals when acquired by oral route (Jawetz et al. , 2001) .Transmission of salmonella infection may take place from one animal to another when they are waiting at slaughterhouse , via faeces in drinking troughs (Gracey and Collins 1999 ). The gastrointestinal tracts of animals and man are common sources of *salmonella* , High protein foods such as meat are most commonly associated with salmonella ( Wanger , 2000 ). *Salmonella* can also be found in the tissues but , may not appear in the faeces ( Bowman et al., 2007 ). However , well cooked meat will be free from *salmonella* . In other hands 21.4% of the whole processed broiler carcasses sampled at processing plants were contaminated with *salmonella* (Jones et al., 1991 ).

Food - borne diseases caused by non - typhoid *salmonella* an important public health problem worldwide .Nearly 1 - 4 million cases of salmonellosis in human occur each year in the United States (David et al 2001 ) . It grow well on meat food ordinary temperature (Gracey and Collins 1999 ) .

The organisms occur more often in the cecum than in any other region of the gut from where they be excreted for varying periods, without the host showing any sign of disease (Morris and Wells ., 1970; Mead, 1982 ;Grau , silliker and Galois ., 1986; Mead, 1989; Zottola and Smith., 1990; Jones et al., 1991) .*Salmonella* from one flock can contaminated another , usually during conditions of intensive rearing and also when there is inadequate cleaning and disinfecting of multi-cage transportation Lorries used convey the birds to the abattoir.

Studies have also shown that poultry transported from the farm often introduce salmonella into the processing plant may result in considerable scattering of salmonellae during processing especially in the plucking machines and scalding tank and may lead to contamination of the final product (McBride et al., 1980; Mead, 1982; Mead, 1989; James et al., 1992).

Poultry slaughter is multi-stage operation and modern plants can process of 200 birds per minute. The major emphasis has been on speedy and cost-effective production with prevention of cross contamination being of less importance, thus, the incidence of carcass contamination with salmonella often exceeds that infection in the level birds (e.g. Notermans et al., 1975; McBride et al., 1980).

The extent of cross-contamination during plucking is governed by the hygiene of scalding process. Alternatives have been developed including simultaneous scalding & plucking & steam scalding. These minimize cross-contamination with *Salmonella spp.*

Intestinal contents can be heavily infected with *Salmonella spp.* (Smith 1969. Quoted in Crabb and Walker 1971). and thus the process of removing the intestine often results in carcass contamination (Bryan et al., 1968).

This process is carried out automatically for chickens, and equipments is calibrated for birds of particular size or weight while every effort is made to standardize this / then are natured variation which can used to damage to the viscera and contamination of carcass with contents Equipments this soiled can transfer organisms to subsequent carcasses, carcasses must be washed after evisceration - spray washing can bring



about significant reduction in numbers of *salmonella spp.* (Bryan et al 1968 ; Morris & Well , 1970 ) .

From a survey of contamination with *Salmonella* which was done in processing plants of two integrated broiler firms. *Salmonella* was found in 33% of samples from live haul trucks and 21.4% of the whole processed broiler carcasses .( Jones , et al ., 1990 ) .

Several studies have concluded that presence of *salmonella spp.* on live poultry can lead to the introduction of organisms into processing plant (Bryan et al., 1968 ;Lahellec and Colin ,1985 ;Bailey et al. ,1990 ;Corry et al. ,2002.) . where the contamination of equipment can result in contamination of the final products (Lillard , 1990 ) . Even spread from slaughterhouse environment back to the farms through contaminated crates and vehicles may be seen , because inadequate cleaning and disinfection result residual fecal soling and live bacteria (Rigby et al 1980 ; Corry et al. ,2002.) .

An important process operation that impacts the presence of microorganisms in poultry slaughter scalding . At present, the trend is to scald poultry at lower temperatures (50 - 52 C<sup>o</sup>), which are more suitable for air-chilled poultry . Lower scalding temperatures may , however , allow some microorganisms including pathogens to survive . A way of avoiding this problem is to use multistage scalding , where poultry is scalded in several poultry surfaces (Berrang et al .,2008 ) .

The next process operation is plucking ,which is closely related to the scalding operation . The main hygienic problem is cross-contamination via equipments or via aerosols in the air . Evisceration is first stage of the clean part of the slaughter process . Consisting of several stages , Evisceration starts with head removal followed by

opening of the body cavity in removal of intestines , and end with cleaning of the carcass (Cox and Pavic 2010 ). From the hygienic point of view , attention is paid to the removal of the intestines and the prevention of cross-contamination with fecal material. The next processing step is chilling which is essential to control microbial growth (James et al, 2006). Common methods include continuous mechanical immersion , chilling and air blast chilling , with or without the incorporation of water sprays to maintain product yield and enhance cooling by evaporation (Mead 2004) It follows from the above overview of basic processing steps in broiler slaughter that there are many steps in the poultry meat processing that could significantly influence the extent of poultry contamination and thus also marketability and incidence of pathogenic microorganisms .The most critical processing steps in this respect include scalding , plucking ,evisceration , and the type of poultry chilling (Keener et al ., 2004 ).

### **1.9.2. *Escherichia coli* : -**

*Escherichia coli* (commonly abbreviated *E.coli* ) is gram - negative , rod-shaped bacterium that is found in the lower intestine of warm-blooded organisms (endotherms) (Q. Ashoton action 2013 ) Most *E.coli* strains are harmless , but some serotypes can cause serious food poisoning in humans , and are occasionally responsible for product recalls due to food contamination (Dippold and vogot ,2005 ). Many strains of *E.coli* are harmless and are found naturally in the gut of humans and animals. Traditionally its presence in foods has been an indication of fecal contamination of food or water . However , particular strains are pathogenic and traveler's diarrhea and haemolytic uremic syndrome (HUS) are caused by *E.coli* strains . Although pathogenic types are rare , in the last few years there have been several food borne outbreaks from

certain strains of *E.coli* . A wide variety of foods have been implicated including unpasteurized apple and orange juices , sprouted seeds , fruit, raw milk cheese ,salads and meat products especially undercooked minced meat patties in hamburgers *E.coli* is killed by heating so cooking food properly is basic method of control . Water can also be source of the bacteria . The harmless strains are part of the normal flora of the gut ,and can benefit their hosts by producing vitamin K<sub>2</sub> ,and by preventing the establishment of pathogenic bacteria within the intestine (Bentley and Meganathan , 1982 ) .

*E.coli* a natural inhabitant of the intestinal tracts of humans & worm - blooded animals , is used as an indicator bacterium because it acquires antimicrobial resistance faster than other conventional bacteria (Miranda et al ,2008 ) . Its presence therefore reliably reflects fecal contamination indicating a possible contamination by enteric pathogenic . Many different types of foods are sources of the bacterium and have been identified as potential sources of shiga toxin producing *Escherichia coli* (STEC ) for which such raw or under cooked foodstuffs get contamination either during primary production (e.g. slaughtering ) or further processing & handling ( cross - contamination during processing human to food contamination via food handlers ) . *E.coli* has been isolated world-wide from poultry meat (Conton et al ., 2008 ; Adesiji et al., 2011 ) , properly due to increased used antimicrobials (Miranda et al., 2008) .

## Chapter Two

### Materials and Methods

#### 2.1 Area study

##### 2.1.1 Sources of samples

The swabs were collected from poultry meat carcasses on slaughterhouse located in Khartoum state Nile East (Automatic abattoir of poultry).

**2.1.2..Collection of samples:-** Total of 60 swabs samples were obtained from carcasses of broiler chickens . Firstly, a total of 30 samples were taken from six critical points CCPs after evisceration, hands of employees , defeathering , after washing , before chilling , after chilling , packing . Secondly , a total of 30 samples were repeated and taken from the same previous six stages CCPs after evisceration , hands of employees , defeathering , after washing , before chilling , after chilling , packing,

A total of 60 Samples were collected in sterile tubes and preserved in ice and transferred to laboratory of Microbiology for culturing.

#### 2.2. Media : -

**2.2.1 Bacterial Culture Media :** Preparation of Blood agar , MacConkey's agar , Nutrient agar , Mannitol salt , Milk agar , DCLS agar. According to Oxiod. Also biochemical analysis were done. (Barrow and Feltham ,2003 .The agar plates were incubated at 37c° for 24 hours

##### 2.2.1.1 A/Solid Media :

**2.2.1.1.1./Blood Agar(oxid)Media:** This is used with blood for Isolation and cultivation of wide variety of fastidious microorganisms

**Table 1: blood agar (oxid) Ingredients**

Ingredients	gm/litre
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0

**2.2.1.1.2./MacConkey's Agar Media:** is commonly used media to culture and indentify gram negative bacilli (especially bacteriaceae.) this media composed of peptone , lactose , bile salt, sodium chloride , neutral red, crystal violet ,agar ,the medium was prepared according to manufacture instruction by dissolving 51.1 gm in distal water , mixed and sterilized by autoclaving at 121c° for 15 minute then distributed into sterile Petri-dish in 25 ml volume , left to solidity and stored at 4 C ° before used .

***MacConkey Agar***

Peptone per liter	20.0g
Agar	15.0g
Na Cl	5.0g
Bile Salts	1.5g
Neutral Red	0.05g
Crystal Violet	1.0g

**2.2.1.1.3./Nutrient Agar Media :** is a basic culture medium used to subculture organisms for maintenance purposes or check the purity of subcultures from isolation plates prior biochemical or serological tests.

**2.2.1.2.B/Semi Solid Media :**

**2.2.1.2.1/Motility Media Table 2: Motility media (oxide)Ingredients**

Ingredients	gm/litre
Beef extract	3.0 g
Pancreatic digest of casein	10.0 g
Sodium chloride	5.0 g
Agar	4.0 g

**2.2.1.2.2./Hugh&Leifsons of Basal Medium**

**Table 3: Hugh&Leifsons of Basal Medium**

Ingredients	gm/litre
Peptone (tryptone)	2.0 g
Sodium chloride	5.0 g
Glucose (or other carbohydrate)	10.0g
Bromthymol blue	0.03 g
Agar	3.0 g
Dipotassium phosphate	0.30 g

**2.2.1.3.c/Liquid media:**

**2.2.1.3.1./Nutrient Broth :**

This is general usage medium for large variety of microorganisms without particular nutritional requirements.

**Table 4: Nutrient Broth Ingredients**

Ingredients	gm/litre
Tryptone	15.0
Meat extract	2.5
Sodium chloride	5.0

**2.2.1.3.2./Peptone water :**

This is used as a growth medium or as the basis of carbohydrate fermentation media whilst a pure culture in peptone water is convenient inoculums for series of fermentation tubes or other diagnostic media

**Table 5 : Peptone water Ingredients**

Ingredients	gm/litre
Peptone	10.0
Sodium chloride	5.0
PH 7.2± 0.2	

**2.2.1.4.d/Selective Media:**

**2.2.1.4.1./ Mannitol Salt Agar(Oxoid) Media:**

This is selective medium for isolation presumptive pathogenic staphylococci, most other bacteria are inhibited , with exception of a few hemophilic species.

**Table 6: Mannitol Salt Ingredients**

Ingredients	gm/litre
' Lab-Lemco' powder	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0

#### 2.2.1.4.2./Milk Palate Agar

**Table 7 : milk Palate Agar Ingredients**

Ingredients	gm/litre
Tryptone	5 g
Yeast Extract	2.5 g
Glucose	1 g
Antibiotic Free Skim Milk	1 g
Agar	10 g

#### 2.2.1.4.3./ DCLS Agar: Deoxycholate Cholate Citrate Lactose Sucrose Agar

Is modified fermentable carbohydrate increase the usefulness medium because non pathogenic Sucrose -fermenting organisms may be recognized by their red colonies e.g Some *Proteus* ,*Enterobacter* & *Klebsiella species*./4/EMB Agar (Eosin Methylene Blue Agar)

Very versatile solid medium , originally developed by Levine for differentiation of *E.coli* and *Aerobacter areogenes* ,it turned out to be



effective for the rapid identification of *Candida Albicans* & was found useful for the identification of *coagulase-positive staphylococci*.

**Table 8 : DCLS Ingredients**

Ingredients	gm/litre
Special peptone	10.0
Sodium citrate	10.5
Sodium thiosulphate	5.0
Lactose	5.0
Sucrose	5.0
Sodium Desoxycholate	2.5
Neutral red	0.03
Special peptone	12.0
Agar	10.0

### **2.3.Bacterial counts:**

#### **2.3.1./Plate Count Agar:**

A medium for the enumeration of culturally microorganisms from water using a colony count technique.

#### **2.3.2..Bacterial Cell Counting Techniques**

This is necessary in diagnosis to enumerate bacterial cells . it is used in diagnostic and food hygiene procedures . Viable bacteria are capable of multiplication with production of visible colonies on or in Agar media , in viable counting method the assumption is made that one well-spaced ,bacterial cells give rise to one colony .Bacteria colonies rather than bacterial cells counted in most of the methods (A non1982).

Viable Bacterial Cell Counting Method .

**2.3.3.Viable Plate counts** (TVC was applied by using serial dilution to each sample (Harrigan and ManCance 1976).

One of the most common methods of the determining cell number is viable plate count . A sample to be counted is diluted in a solution that will not harm the microbe , yet does not support its growth .

A standard plate count method used to determine the number of viable bacterial cells per unit volume of sample , to determine the number of viable bacterial cells per milliliter of liquids sample a fixed volume of sample transferred to a plate , the solution spread across the plates and the colonies counted after incubation . the colonies were referred to as colony forming unites (CFUs). on the plate was determined , it was divided by the volume plated determined the concentration of cells in the sample , the sample contained over one thousand cells per unit volume then it produced to many CFUs to a count accurate on the plate. These samples diluted in sterile media before was transfered to plate media so that a countable number of colonies appeared ,since the actual concentration of the sample was unknown it was common practice to dilute the sample serially (for example)the spread -plates the multiple serial dilutions produced the lowest number of CFUs , the plate with countable number of colonies was selected to count when using standard size Petri - dishes ,a countable plate was one with between 30 and 300 CFUs .Dilutions with fewer than 30 colonies are easy count , dilution determined by dividing the amount plated . Once the concentration of the cells at the specific dilution was determined , the concentration in the original sample WAS calculated after dividing by the total dilution (using Aseptic technique ).

## **2.5.Procedures of microbial analysis : -**

The samples were put in nutrient broth in test tubes , then 5ml of this solution incubated for 18 -24 hours at 37c° ( overnight ), for bacterial growth . Firstly serial dilutions prepared from Normal saline solution included bacteria to be dilute Second LY serial folds dilution in sterile test tubes , every one contain 9 ml normal saline were prepared . Then

1 ml by micro pipette from nutrient broth included bacteria ,added in first tube 9 ml of normal saline ,dilutee 1.From first tube dilution 1/10 had taken 1 ml by micro pipette added to second tube included 9ml normal saline .dilution 1/100 concentration , till the dilutions reached 1/100000.concentration . From dilution 4th tube (1/10000 ) had taken 1ml by micro pipette , then added to surface of Petri - dish contain nutrient Agar Media or count plate , incubated ( overnight ) at 37 °C for 18-24 hours. And show colonies . Duplicated this test at dilution 5th (1/100000 ) on Petri - dish .or count plate . incubated overnight at 37 °C for 24 hours. Show colonies .This methods called ( Mile and Misera ) . Then the colonies counted .

**2.6.Colonies Isolation :** According to Cultural Media preparation Oxiod (2003).

1/ *Salmonella* isolated in ManCconkey Media : Pale yellow non lactose ferment colonies

2/*Escherichia coli* isolated in ManCconkey Media , *E.coli* fermenting Lactose ( pink color ), *E. coli* not fermenting Lactose ( color less ).

### **2.6.1.Salmonella :**

Classification :

Family : *Enterobacteriaceae* Spp : *S. typhi* ,*S. para typhi* A,*S. para typhi* B ,*S. paratyphi* C ,*S. typhi* murium .

Genus *Salmonella* classified into two species : *S. enterica* and *S. bongori*.

*S. enterica* consist subspecies: *S. enterica* , *S. salamae* ,*S. arizonae* ,*S. diarizonae* ,*S. houtenae* , *S. indica*.

In poultry two species:

1/ *Salmonella Gallinarium*

2/ *Salmonella Pullorum* . Morris (2013).

Definition : Gram stain : Gram negative facultative anaerobic mobile short rods or non spore-forming rods that ferment glucose, usually with gas,

Microscopic Examination . Gram stain : Gram negative . Motility : motile

Culture : ManCconkey Media : Pale yellow non lactose ferment colonies

D.C.A. : Large pale yellow non lactose or sucrose not ferment with black center colonies

C.L.E.D. : Blue non lactose ferment colonies .

### **2.6.2. *Escherichia coli* :**

**Classification** : Domain : Bacteria . Kingdom : *Eubacteria* . Phylum : *Proteobacteria* . Class : *Gamma proteobacteria* . Order : *Enterobacteriales* . Family : *Enterobacteriaceae* . Species : *Coli* . Nominal name *Escherichia coli* . Genus ; *Escherichia* .

1/*Enterohemorrhagic E.coli* (EHEC)

2/*Enterotaxigenic E.coli* (ETEC)

3/*Entropathogenic E.coli* (EPEC)

4/*Entroaggrative E.coli* (EAEC)

5/*Entroinvasive E.coli* (EIEC) Morris (2013) .

*Escherichia coli* gram stain lactose fermented , Non -spore - forming bacilli grow in both Aerobically and Anaerobically . they do not ferment mesoitol ,they give negative reactions in Voges-Proskauer.Motility : Motile .Culture : D.C.A : pink and opaque MacConkey Agar pale - yellow

## **2.7..Biochemical Tests :**

### **2.7.1Primary tests :**

#### **2.7.1.1The Catalase test :**

It detects the bacteria which are catalase - positive due to presence of catalase enzyme by hydrogen peroxide .

**2.7.1.2.The Oxidase Test :** It detects the presence of cytochrome oxidase enzyme called indophenole oxidase , it reduced colorless reagent becomes an oxidized colored product. Oxidation - Fermentation Test :

It is used to determine if gram - negative bacteria metabolize carbohydrates oxidatively , by fermentation .and therefore have no ability to use the carbohydrate in the media .Bacterial Motility tests :Hanging drop method of motility : under observation immersion oil in high power lens motility is characterized by fast unidirectional movement as compared to the Brownian motion whereby the cells move round in one particular point .

**2.7.2.Secondary tests :2.7.2.1/ Indole Test** : Test (Indole Methyl - Red ,Voges - Proskauer ,and Citrate ) that are used to differentiate among the Gram Negative bacilli in the family Enterobacteriaceae .positive (Cherry red coloration in oil layer on top of broth ) . Negative ( Not red in oil layer on top of broth

**2.7.2 2/ Citrate Test** : A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism .The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator .The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates .

**2.7.2.3/Urease Test** :This test is performed selective agar medium .a purity plate must be included . To check for purity of organism . Inoculate slope heavily over the entire surface and stab with loop/nichrome wire. Incubate inoculated slope at 35-37c° in a water bath , Examine slope after 4 hr and after overnight incubation . Positive Result : Purple / Pink color .

Negative Result : Color of medium remains unchanged

**Tables Biochemical Tests for identification of *Salmonella spp.* and *Escherichia coli* (Primary and Secondary Tests)**

**.There Table 9 : Primary test**

Type of bacteria	Oxidase test	Catalase Test	OF test	Sugar test	Motility Test
<i>Escherichia coli</i>	-	+	F	+	Motile

<i>Salmonella</i>	-	+	F	+	Motile
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OF=Oxidation & Fermentation

**Table 10: Secondary test**

Type of bacteria	Indole (Kovac-s) test	Urease Test	Citrate Test	KIA(kilger Iron Agar )tests			
				Slope	H <sub>2</sub> s	Butt	Gas
<i>Escherichia coli</i>	+	-	-	yellow	yellow	-	+
<i>Salmonella</i>	-	-	+	Red	yellow	+	+

## Chapter Three

### Results

The study revealed Isolation and Identification of two types of bacteria at Different Operational Points . *Salmonella spp.* and *Escherichia coli* . in ( table 11).

Isolation and Identification of bacteria at different operational points under investigation revealed at defeathering stage: load of contamination was 11.18% (Fig1) for *Salmonella spp.* , and load of contamination in this stage 7.42% (Fig 2) for *Escherichia coli* as highest level of contamination where at the chilling stage the load contamination 16.66% of *Salmonella spp.* and 1.85% for *Escherichia coli* as lower level contamination. (Figure 3)

Figure 1

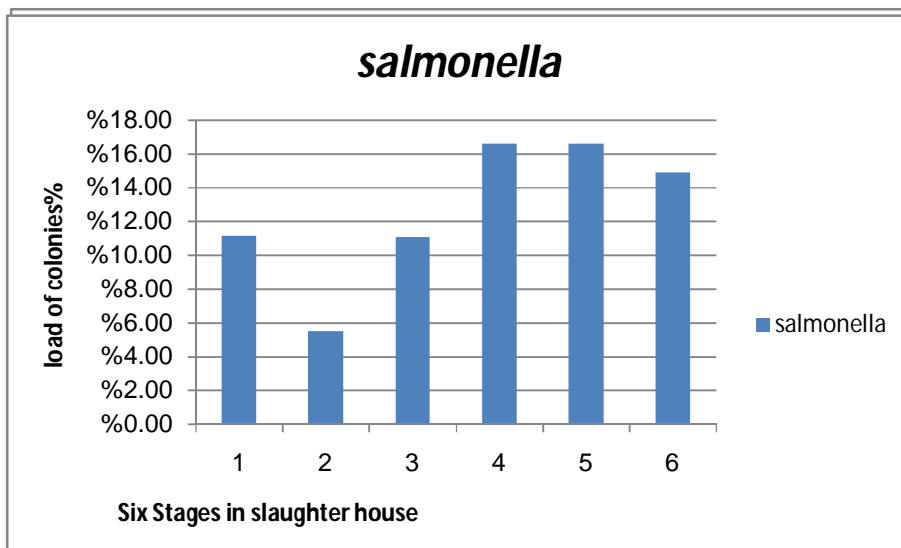
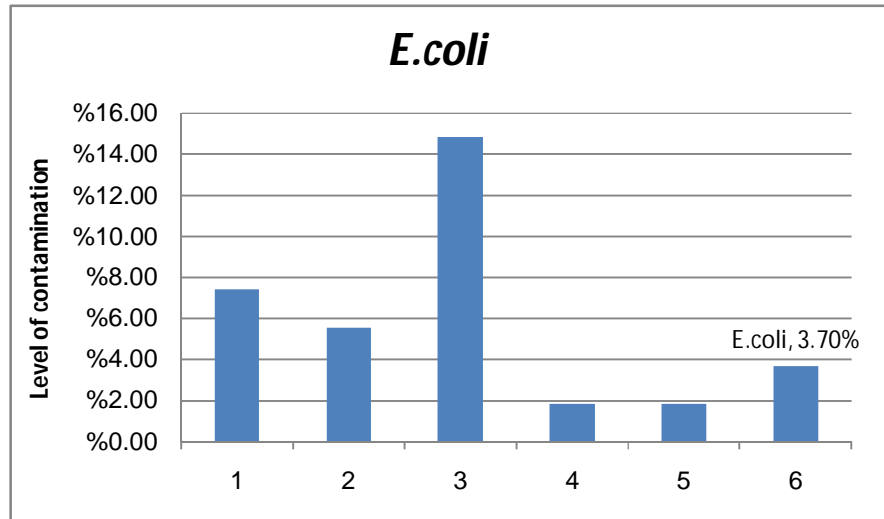




figure 2



Loaf of contamination in Six stages in slaughter house

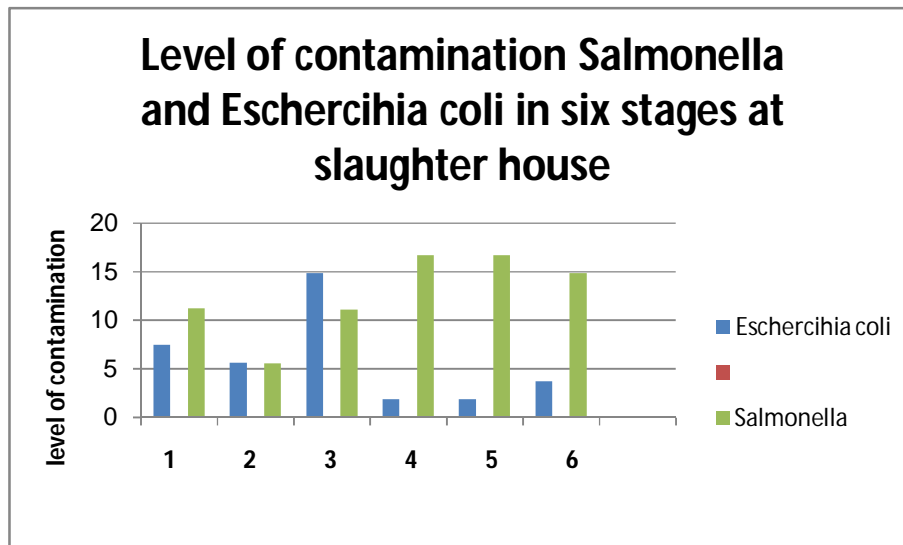
- 1/Defeathering stage
- 2/Evisceration stage
- 3/ Employees stage
- 4/After Washing stage
- 5/After chilling stage
- 6/After Packing

**Table 11: Numbers and percentage of *Salmonella spp.* and *Escherichia coli* Isolated and, Identified at different operational points in poultry slaughterhouse(10 samples collected stage each stage :**

Points	<i>Salmonella SPP.</i>	<i>Escherichia coli</i>	Total
Defeathering	11.18%	7.42%	18.60%

Evisceration		5.5%		5.55%	11.05%
Empolyees		11.11%		14.82%	14.82%
After Washing		16.66%		1.85	18.51%
After Chilling		16.66%		1.85%	18.51%
Packing		14.81%		3.70%	18.51%
Total		75.92%		24.08%	100.00%

Figure 3



## Statistical Analysis

The data were analyzed with Statistical Package for Social Sciences (SPSS), version software (SSPS Inc, and Chicago IL, USA). All bacterial counts were converted to log<sub>10</sub> cfu/ml (g) for analysis and ANOVA was performed. Statistical significance was set at a p-value of  $p \leq 0.05$ .

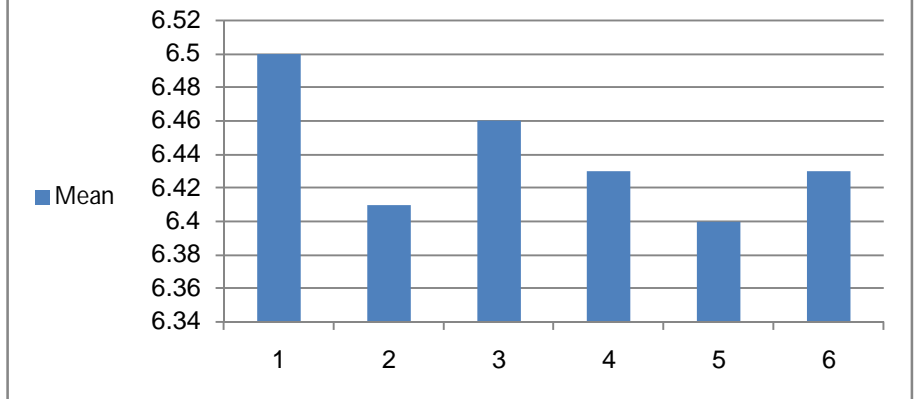
The study revealed a statistically Significant Difference at ( $P \leq 0.05$ ) after Defeathering and after Chilling as shown table 12.

**Table12:** Comparison of mean Total Viable Counts of bacteria(log<sub>10</sub> CFU(m<sup>-2</sup>), ±Standard Deviation at different operational points. As shown, the TVC revealed the highest contamination at defeathering point Mean 6.50 and Std. Deviation ±0.09. and lowest contamination after chilling point Mean 6.40 at Std Deviation ±0.16 (Fig. 4).

**Table 12 : Mean and Standard Deviation of Total Viable Counts of Bacteria(Log<sub>10</sub>cfu/cm<sup>2</sup>) at Different Operational Points**

Points	Mean(Log <sub>10</sub> cfu/cm <sup>2</sup> )	Standard Deviation	Significant Difference
Defeathering	6.50	±0.17	**
Evisceration	6.41	±0.08	**
Employees	6.46	±0.09	**
After washing	6.43	±0.12	**
After chilling	6.40	±0.16	**
During Packing	6.43	±0.12	**

### Mean of Salmonella and Escherichia coli in six stages in slaughter house



## Chapter Four

### DISCUSSION

In this study the mean bacterial viable count ( TVCs ) obtained from the result showed lower contamination after chilling stage , and highest contamination at defeathering stage . This data in accordance to the finding of Mead ( 2004 ) who reported that substantial decrease in TVCs Contamination may occur due to bacterial population associated with water from the scald tank , rubber fingers at the exit of defeathering machine Georanras et al.,( 1997 ). Feathers generally may contaminate external surface of the carcass skin during early processing stages . The highest level of viable aerobic bacteria recovered from the samples . The result in agreement with the findings of Ramires et, al( 1997) and Hinton et al.,( 2000). who reported that broiler carcass can contaminated by bacteria when contact with ingesta or feces from alminatory tract during grow - out . ) and Coliform bacteria counts after washing & after chilling , Drewnaik et al ., (1984) found that there was build up 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 water decreases the bacteria present on the skin of poultry. Defeathering or picking achieved by passing the birds through rows of rotating rubber fingers that remove the feathers and help squeeze the remaining blood. mechanical pickers and other items used for processing must be constructed to ensure clean lines Houston ( 1985 ). It represents another chance for cross - contamination , consider microorganism like *Salmonella* have been shown to attach firmly to poultry skin and rubber fingers act as transmitters for contamination . The extent of cross - contamination during plucking is governed by the hygiene of scalding process. Alternatives have been developed including

simultaneous scalding and plucking and steam scalding (These minimize cross - contamination with *Salmonella* spp.

*Salmonella* are more frequently isolated from carcasses after defeathering than following any other processing operation McBride et.al (1980). Following hot or hard scalding , defeathering damages &removes the epidermal layer and exposes a new surface layer .

Contamination either during primary production (e.g. slaughtering ) or further processing and handling ( cross - contamination during processing human to food contamination via food handlers ) . *Escherichia Coli* has been isolated world-wide from at (Contamination of poultry properly due to increased used antimicrobials ( Miranda et al,men et al , 2008 ; Adesiji et al . 2011 ). Also due 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.

On study of the affect of processing procedures and overall environmental and hygienic condition of the microbiological quality and safety found heavily contamination at scalding and defeathering with *Salmonella and Escherichia coli* (Abu-Ruwida et al., 1994 ). this data in agreement with the data of this study .

The presence of *Escherichia coli* in fresh meat can be attributed to carcass contamination with the gastrointestinal content during processing .The contamination levels recorded in the point of washing in all sites carcasses may be due to unclean management during the washing , this agree with Ali ( 2007) which is in agreement with the data in this study, because there was also contamination in this stage (table 11).

In this study, the Mean TVCs obtained from chicken carcasses in following CCPs ; after defeathering, after evisceration , after spray water

and hands of employees are similar to that obtained by Kabour (2011). Furthermore , the Mean TVCs are confirming the reports of Mohamed Noor et al., (2012) . who found the same TVCs of some CCPs including : after defeathering , after evisceration , after chilling and employees hands at the same time.

### **Conclusions:**

The obvious conclusion to be drawn from the results is that:

- 1/ There is contamination in an automatic poultry slaughterhouse in Khartoum State .
- 2/ *Salmonella species and Escherichia coli* were isolated from poultry meat at all stages of processing.
- 3/ The highest contamination was shown at defeathering stage. and lowest contamination after chilling stage.

### **Recommendation:**

- 1/ It is important to Apply HACCP System , based on the use of multi - functional strategies (sanitizers & modern disinfections techniques ).to reduce bacterial contamination.
- 2/ Consider personnel hygiene ,hands gloves , masks, head cover ,contaminated equipment cross-contamination from raw material as plucked fingers during defeathering stage. ,valves or plates.
- 3/ Proper chilling and freezing of poultry meat prevent multiplication microorganisms.

4/ Cooking at high temperatures of 100c° will help to eliminate pathogen before consumption.



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59