Sudan University of Science and Technology College

Of Graduate Studies

Investigation of Bacterial Load and Contamination of Automatic and Traditional Poultry Slaughtering Processes by *Salmonella spp., Escherichia coli and Staphylococcus aureus* in Khartoum State - Sudan

تقصي المحتوي البكتيرى والتلوث في عمليات ذبيح الدواجن بالمجزر الالي و التقليدي بواسطة بكتريا السالمونيلا والاشريكية القولونية والمكورات العنقودية في ولاية الخرطوم- السودان

A Thesis Submitted to the College of Graduate Studies in Fulfillment of the Requirement of Attaining PhD in Veterinary Preventive Medicine (Food Safety)

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Dedication

To the soul of my father and mother (may be merciful to them) who dedicated all their efforts to my best.

To my wife Dr. Maha

To my beloved (sons and daughters)

I dedicate this work.

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Praise be to Allah who gave me the strength and patience to conduct this research.

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ABASTRACT

This study revealed isolation and identification of three types of bacteria at six different operational processes (*Salmonella spp., Escherichia coli and Staphylococcus aureus)* in two types of operations, Automatic poultry slaughter house and Traditional poultry slaughtering process in Khartoum State.

180 swab samples were collected from carcasses of broiler chicken at six stages (Scalding, defeathering, evisceration, after washing, after chilling and hands of Employees.) during slaughtering process in Automatic poultry slaughter house and Traditional poultry slaughtering process.

The results revealed that the highest contamination was detected in the traditional poultry slaughtering processes and lowest contamination was detected in an automatic poultry house process. The scalding point showed high contamination. (TVC) (Mean (log_{10}CFU/ml**)** 7.28 ±0.11) in traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 7.01 ±0.21) in an automatic poultry slaughter house.

Defeathering point showed high contamination (mean (log_{10}CFU/ml**)** 7.43 ±0.08) in traditional slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 7.09 ±0.13) in an automatic poultry slaughter house.

Evisceration point showed high contamination (mean (log_{10}CFU/ml**)** 7.43 ±0.16) in traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 6.86 ±0.15) in an automatic poultry slaughter house.

After washing point showed high contamination (mean (log_{10}CFU/ml**)** 7.36 ±0.11) in Traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 6.68 ±0.15) in an automatic poultry slaughter house.

After chilling point showed high contamination (mean (log_{10}CFU/ml**)** 7.30 ±0.14) in traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 6.84 ±0.29) in an automatic poultry slaughter house.

Employee's hands showed high contamination (mean (log_{10}CFU/ml**)** 7.37 ±0.16) in traditional poultry slaughtering process and low contamination (mean (mean (log_{10}CFU/ml**)** 6.74 ±0.18) in an automatic poultry slaughter house.

The results of samples cultured growth of intended bacteria in Different Operational Points in automatic slaughter house is (*Salmonella spp., Escherichia coli and Staphylococcus aureus***)** at Scalding stage the numbers of positive samples was as follows 12 (10.4%) samples were positive for *Salmonella spp.*, 3(2.6%) for *Escherichia coli.spp., and* 2(1.8%) samples for *Staphylococcus aureus.*

At Defeathering stage the numbers of positive samples was as follows 12(10.4%) samples were positive for *Salmonella spp., 4*(3.5%) for *Escherichia coli.spp, and* 11(9.6%) for *Staphylococcus aureus*

At Evisceration stage the numbers of positive samples was as follows 15 (13%) samples were positive for *Salmonella spp.*, 1(0.9%) for Escherichia *coli.spp, and* 1(0.9%) for *Staphylococcus aureus.*

At After Washing stage the numbers of positive samples was as follows 15(13%) samples were positive for *Salmonella spp.*, 3(2.6%) for *Escherichia coli.spp, 2*(1.8%) for *Staphylococcus aureus*

At After Chilling the numbers of positive samples was as follows 15(13%) samples were positive for *Salmonella spp.*, 1(0.9%) for *Escherichia coli. and1* (0.9%) for *Staphylococcus aureus*

At Employees hands the numbers of positive samples was as follows 13 (11.3%) samples for *Salmonella spp.*, 2(1.7%) for *Escherichia coli.spp, and* 2(1.8%) for Staphylococcus *aureus.*

The results showed the number of positive samples was in Different Operational Points in traditional poultry slaughtering processes (*Salmonella spp., Escherichia coli and Staphylococcus aureu***)** scalding stage the numbers of positive samples was as follows 14(8%) samples were positive for *Salmonella spp.*, 6(3.4%) for *Escherichia coli, and 9*(5.1%) for *Staphylococcus aureus.*

At Defeathering the numbers of positive samples was as follows 12(6.9%) samples were positive for *Salmonella spp.,* 8(4.6%) for *Escherichia coli, and* 6(3.4%) for *Staphylococcus aureus.*

At Evisceration the numbers of positive samples was as follows 12(6.9%) samples were positive for *Salmonella spp.,* 10(5.7%) for *Escherichia coli, and* 8(4.6%) for *Staphylococcus aureus.*

At After Washing the numbers of positive samples was as follows 12(6.9%) samples were positive for *Salmonella spp., 3*(1.07%) for *Escherichia coli, and* 10 (5.7%) for *Staphylococcus aureus.*

After Chilling the numbers of positive samples was as follows *14*(7.3%) samples were positive for *Salmonella spp.,* 7(4%) for *Escherichia coli, and 10*(5.7%) for *Staphylococcus aureus.*

At Employees hands the numbers of positive samples was as follows 12 (6.9%) samples were positive for *Salmonella spp., 10* (5.7%) for *Escherichia coli, and12* (6.9%) for *Staphylococcus aureus*

The statistical analysis of results revealed that there was significant difference at P-Value ($P \leq 0.01$) in all Different Operational Points in two types of an automatic slaughter poultry house and traditional poultry slaughtering process in Khartoum state.

Also the results cleared that, the highest contamination level was detected at Evisceration in the Traditional poultry Slaughtering process (mean(log_{10}CFU/ml**)** 7.43 ±0.16)) and the numbers of positive samples was as follows 12(6.9%) samples were positive for *Salmonella spp.,* 10(5.7%) samples were positive for *Escherichia coli, and* 8(4.6%) samples were positive for *Staphylococcus aureus,* the low level contamination was detected after Chilling which *showed* (mean(log_{10}CFU/ml**)** 7.30 ±0.14) and the numbers of positive samples was as

follows 14(7.3%) samples were positive for *Salmonella spp.,* 7(4%) samples were positive for *Escherichia coli and* 10(5.7%) samples were positive for *Staphylococcus aureus*. While on the automatic poultry slaughter house the highest contamination level at Evisceration showed **(** mean(log_{10}CFU/ml**)** 6.86 ±0.15) and the numbers of positive samples was as follows 15(13 %) samples were positive for *Salmonella* , 1(0.9%) samples were positive for *Escherichia coli* and 1(0.9%) samples were positive for *Staphylococcus aureus,* the lowest contamination after Washing showed (mean(log_{10}CFU/ml**)** 6.68 ±0.15) and the number of positive samples was as follows 15(13%) samples were positive for *Salmonella spp.* 3(2.6%) samples were positive for *Escherichia coli.spp. and* 2(1.8%) samples were positive for *Staphylococcus aureus.*

Statistically, there was significant difference at P-Value ($P \leq 0.01$) among six stages (Scalding, defeathering, evisceration, after washing, after chilling, hands of Employees.) from results the of *Salmonella spp., Escherichia coli and Staphylococcus aureus* were predominant in traditional poultry slaughtering process and at an automatic abattoir processing that affected on safety and quality of poultry meat in Khartoum State . Concerning of HACCP System which was reduced bacterial contamination.

ملخص الاطروحة

اظهرت هذه الدراسة عزل ومعرفة ثلاثة انواع من البكتريا وهي(بكتريا السالمونيلا وبكتريا الاشريكيةالقلونية وبكتريا المكورات العنقودية الذهبية) في عدد ست نقاط لعمليات ذبيح لحوم الدواجن في المجازر التقليدية والمجازر الالية في ولاية الخرطوم.

جمعت عدد 180 (مسحات) في ست نقاط من لحوم الدواجن خلال مراحل عمليات الذبيح في كل من النوعين وهي مراحل(السمت ونزع الريش وتفريغ الاحشاء وبعد الغسيل وبعد التبريد ومن ايادي العمال.)

اجريت التجارب لعزل والتعرف علي بكتريا السالمونيلا وبكتريا الاشريكيةالقلونية وبكتريا المكورات العنقودية الذهبية في مراحل عمليات الذبيح في المجازر الالية والتقليدية

واظهرت النتائج مستوى عالي للتلوث مسببا ببكتريا السالمونيلا وبكتريا الاشريكيةالقلونية وبكتريا المكورات العنقودية الذهبية في النقاط الستة الحرجة للمجازر التقليدية مقارنة للمجازر الالية بنفس البكتريا. كذلك اظهرت النتائج ارتفاع عالي للتلوث للعد البكتري في عمليات الذبيح في المجازر التقليديه وكان المتوسط والانحراف المعياري عند مرحلة السمت : (0.21± 7 .01) واقل انخفاض للتلوث في المجزر الالي) (0.11± 7.28) و(0.08± 7.43) في مرحلة نتف الريش اعلي ارتفاع للتلوث في عمليات الذبيح في المجزر التقليدي (0.13± 7.09) واقل انخفاض للتلوث في المجزر الالي(0.16± 7.43) في مرحلة تفريغ الاحشاء اعلي ارتفاع للتلوث في عمليات الذبيح في المجزر التقليدي (0.15± 6.86) واقل انخفاض للتلوث في المجزر الالي (0.11± 7.36) و في مرحلة الغسيل اعلي ارتفاع للتلوث في عمليات الذبيح في المجزر التقليدي (0.15± 6.68) واقل انخفاض للتلوث في المجزر الالي(0.14± 7.30) في مرحلة التبريد اعلي ارتفاع للتلوث في عمليات الذبيح في المجزر التقليدي(0.29± 6.84) واقل انخفاض للتلوث في المجزر الالي (0.16± 7.37) في مرحلة ايادي العمال اعلي ارتفاع للتلوث في عمليات الذبيح في المجزر التقليدي (0.18± 6.74) واقل انخفاض للتلوث في المجزر الالي اظهرت النتائج اعداد ونسب العزلات الموجبة في المراحل المختلفة لعمليات الذبيح المجزرالالي (بكتريا السالمونيلا و بكتريا الاشريكية القلونية و المكورات العنقودية الذهبية) في مرحلة السمت: (10.4%) 12 بكتريا السالمونيلا(2.6%)3 بكتريا الاشريكيةالقلونية(1.8%)2 بكترياالمكورات العنقودية الذهبية في مرحلة نتف الريش(10.4%)12 بكتريا السالمونيلا(3.5%)*4* بكتريا الاشريكية القلونية (9.6%)11بكترياالمكورات العنقودية الذهبية في مرحلة تفريغ الاحشاء(13%) 15 بكتريا السالمونيلا(0.9%)1 بكتريا

الاشريكيةالقلونية (0.9%)1بكترياالمكورات العنقودية الذهبية في مرحلة الغسيل(13%)15 بكتريا السالمونيلا(2.6%)3 بكتريا الاشريكيةالقلونية (1.8%)*2*بكترياالمكورات العنقودية الذهبية في مرحلة التبريد(13%)15 بكتريا السالمونيلا(0.9%)1 بكتريا الاشريكيةالقلونية (0.9%)1بكترياالمكورات العنقودية الذهبية في مرحلة ايادي العمال(11.3%) 13 بكتريا السالمونيلا(1.7%)2 بكتريا الاشريكيةالقلونية (1.8%)2بكترياالمكورات العنقودية الذهبية.

اظهرت النتائج اعداد ونسب العزلات الموجبة في المراحل المختلفة لعمليات الذبيح في المجزرالتقليدي(بكتريا السالمونيلا و بكتريا الاشريكيةالقلونية و المكورات العنقودية الذهبية) في مرحلة السمت:(8%)14 بكتريا السالمونيلا(3.4%)6 بكتريا الاشريكيةالقلونية (5.1%)*9* بكترياالمكورات العنقودية الذهبية في مرحلة نتف الريش(6.9%)12 بكتريا السالمونيلا(4.6%)8 بكتريا الاشريكيةالقلونية (3.4%)6بكترياالمكورات العنقودية الذهبية في مرحلة تفريغ الاحشاء(6.9%)12 بكتريا السالمونيلا(5.7%)10 بكتريا الاشريكيةالقلونية (4.6%)8 بكترياالمكورات العنقودية الذهبية في مرحلة الغسيل(6.9%)12 بكتريا السالمونيلا(1.07%)*3* بكتريا الاشريكيةالقلونية (5.7%) 10بكترياالمكورات العنقودية الذهبية في مرحلة التبريد (7.3%)*14* بكتريا السالمونيلا(4%)7 بكتريا الاشريكيةالقلونية(5.7%)*10* بكترياالمكورات العنقودية الذهبية في مرحلة ايادي العمال(6.9%) 12 بكتريا السالمونيلا(5.7%) *10* بكتريا الاشريكيةالقلونية *12* (6.9%) بكترياالمكورات العنقودية الذهبية.

ان التحليل الاحصائي للنتائج اظهر ان هنالك فروق معنوية لكل مراحل الذبيح المختلفة عند القيمة ($p\geq0.1$) في المجزر الالي والمجزر التقليدي في ولاية الخرطوم

وكذلك اوضحت النتائج ان اعلي مستوي للتلوث في المجزر التقليدي عند مرحلة تفريغ الاحشاء وكان المتوسط 7,43 والانحراف المعياري 0.16± والعد البكتري :(6.9%)12 بكتريا السالمونيلا (5.7%)10 بكتريا الاشريكيةالقلونية(4.6%)8 المكورات العنقودية الذهبية واقل تلوثا عند مرحلة بعد التبريد وكان المتوسط7,30 والانحراف المعياري0,14± والعد البكتري: بكتريا السالمونيلا 14(7.3%) وبكتريا الاشريكيةالقلونية10(5.7%) وبكتريا المكورات العنقودية الذهبية8(4,6%) بينما في المجزر الالي كان تلوثا اعلا عند مرحلة تفريغ الاحشاء وكان المتوسط6,86 والانجراف المعياري 0,15± و كان العد البكتري: بكتريا السالمونيلا15(13%)

وبكتريا الاشريكيةالقلونيه1(0,9%) وبكتريا المكورات العنقودية الذهبية1(0,9%) واقل تلوثا عند مرحلة بعد الغسيل وكان المتوسط6,78 والانحراف المعياري 0,15± وكان العد البكتري بكتريا السالمونيلا15(13%) وبكتريا الاشريكيةالقلونية 3(2,6%) وبكتريا المكورات العنقودية الذهبية 2 (1,8%).

وبالتحليل الاحصائي كانت توجد فروق معنوية عند القيمة($p\geq0.1$) في كل المراحل الستة لكل الثلاثه انواع من البكتريا بكتريا السالمونيلاوبكتريا الاشريكيةالقلونية وبكتريا المكورات العنقودية الذهبية

وكانت سائدة عند كل مراحل عمليات الذبيح في الذبيح التقليدي والالي مما تؤثرعلى سلامة وجودة لحوم الدواجن .لذا تطبيق برنامج وتحليل المخاطر والتحكم في النقاط الحرجه يقلل من التلوث البكتري

IIX

INTRODUCTION

Poultry industries in Sudan began in1926 by enter a group of Wyandotte 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 (Ministry of Animal Resourcesand Fisheries). According to field survey in 2009 the production of broilers was 17.5 million chicks, and the poultry factories in Khartoum state, were about 10 factories of poultry broilers production with capacity of 25000 tons /hour. Nagla (1998) .

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 sever 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 aureus* and other bacteria (Mead etal1994).

In recent years, some highly publicized outbreaks of food borne disease caused by pathogenic bacteria, such as *Escherichia coli O157:H7*, have increased consumer concerns and interest in food safety. As a result, regulatory authorities and the industry have undertaken efforts to improve sanitary conditions and the microbiological quality of meat and poultry. Actions taken by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) include the "Cattle Clean Meat Program" and the "Zero Tolerance" policy of 1993, which instructed inspectors to enforce the requirement of knife-trimming for removal of all visible physical contaminants from carcasses prior to washing and chilling; and the enforcement of new inspection regulations for meat and poultry, which require establishment of sanitation standard operating procedures, operation under the hazard analysis critical control point (HACCP) food safety management system, and establishment of microbiological performance criteria and standards for Escherichia coli biotype I and Salmonella levels of contamination as a means of verifying proper application of HACCP.

Poultry meat is essential part of animal food market and its production is increasing tosatisfy the public demand world-wide as relatively in expensive cost if compared with other animal protein sources (Bryan 1980., Anand etal, 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 (Keener et al; Kabour 2011).

Skin of poultry carcasses always exposed to high average rate of microorganisms, they can pathogenic that cause food-borne illness as well asfood spoilage, they series

of microorganisms on the surface of carcasses which can be canalized in order to indicate the microbial quality , the level of hygiene in production and handling and the correctmaintence of cold chain (Sandron and Arvanitoyannis,1999) .These systems present some advantages over traditionalmethods,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 0pporturnity exists for contamination of carcasses by microorganisms from the processing plant by cross contamination from the birds , numbers of bacteria or carcass surfaces vary considerablyby different stage of processing (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 at defearthening the microorganisms are widely distributed under normal circumstances and are spread over the skin during scalding and defeathering on inner and outersurfaces duringevisceration of the further processing(Bailcy et al, 1987).Quality of poultry meat during slaughtering and packing andhygienic statute ofslaughterhouse (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 etal, 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 0.157H7 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 , ateach stage of process, opportunity exists for contamination from other birds , numbers of bacteria on carcass surfaces vary considerably by different stage of processing (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).

Hazard Analysis and Control of Critical Points (HACCP) in poultry is extremely important because it involves the constant monitoringof 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, and Galhardo et al 2006).

A HACCP principle for control of the microbiological quality of intended foods to human's consumption (FA0 2012). one the most important parameters intervening in biosecurity 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).

Hazard Analysis and Control of Critical Points (HACCP) is a systematic method that serves as the foundation for assuring food safety in the modern world. HACCP is designed to control food borne hazards form production, through manufacturing, storage and distribution of food products. HACCP originated in the late 1950s when NASA required food at the highest safety level for manned space flight and the system was developed in order to achieve this objective by the Pillsbury Company. The first public showing of an early form of HACCP took place in 1971 during the National Conference of Food Protection. Since that time, when there were only 3 principles, HACCP has developed and it is now defined as consisting of 5 preliminary steps and 7 principles. However, the basic philosophy of HACCP remains the same in that it does not rely on end product testing to ensure that the food is safe for consumer but instead builds food safety into the product through the manufacturing process. There is an increase in demand for foods to be safe by consumers and this, in recent years, relates as much to additives and allergens as it does to microbial contamination. This demand has lead food processing companies to develop food safety management systems which are based on or built around HACCP. There are numerous approaches world-wide including guidelines produced by the NACMCF, National Standards and other systems such as the BRC Global Standard and ISO 22000:2005.

Food safety is linked to the presence of food-borne hazards in food at the point of consumption. Since food safety hazards can occur at any stage in the food chain it is essential that adequate control be in place. Therefore, a combined effort of all parties through the food chain is required. For this reasons many different food standards have been developed. On the other hand ISO 9001 isstandard for quality management (QM) systems for different types of production or business. The so called food standards are standards for managing quality and food safety in food business or in whole food chain. ISO 9001:2000 is maintained by the International Organization for Standardization (ISO) and is administered by accreditation and certification bodies. ISO 9001:2000 specifies requirements for a QM system where an organization needs to demonstrate its ability to consistently provide product that meets customer and applicable regulatory requirements, and aims to enhance customer satisfaction through the effective application of the system. These include processes for continual improvement of the system and the assurance of conformity to customer and applicable regulatory requirements. All requirements of

International Standard are generic and are intended to be applicable to all organizations, regardless of type, size and product provided. ISO 22000:2005 is an internationally recognized standard intended to harmonize on a global level the requirements for food safety management within the food chain. It has been designed to be compatible with other management system standards such as ISO 9001 and can be implemented within an integrated management system. The standard combines the key elements to enable management of food safety along the food chain including: integrating the principles of HACCP and application steps developed by Codex Alimentarius Commission; system management; control of food safety hazards through pre-requisite programmes and HACCP plans; interactive communication with suppliers, customers, regulators, consumers; continual improvement and updating of the management system. The British Retail Consortium (BRC) Standard was created to establish a standard for the supply of food products and to act as key piece of evidence for UK retailers and brand owners to demonstrate due diligence (taking all reasonable precautions to prevent an unsafe or illegal product causing customer illness or injury) in the face of potential prosecution by the enforcement authorities. Certification to the BRC standard verifies technical competence and aids manufacturers, brand owners and retailers fulfilment of legal obligations. It also safeguards the consumer. This standard possesses a comprehensive scope covering all areas of product safety and legality, addresses part of the due diligence requirements of both the supplier and the retailer. It covers such critical topics as: HACCP system, QM, factory environment standard, and product and process control. GunWirtanen and Satu Salo, et al (2007).

Objectives:-

- 1- To evaluate the total bacterial load on poultry meat during slaughtering process in automatic abattoir and Traditional slaughter processes.
- 2- To isolate and Indentify *Salmonella spp.*, *Escherichia Coli and Staphylococcus aureus at* different points during slaughtering processes
- 3-To compare the Total Bacterial counts and isolation of *Salmonella spp.*, *Escherichia Coli and Staphylococcus aureus* in automatic abattoir, and Traditional slaughter processes.
- 4-To detect the critical points, which need to be controlled to reduce contamination in poultry automatic abattoir, and Traditional slaughter processes.
- 5. To indentify microbial contamination point for establishing the critical control points (CCP) in poultry automatic slaughterhouse, and Traditional slaughter processes.

Chapter One

Literature Review

2.1: Poultry Slaughtering house Processing: -

Obtaining poultry meat is a similar process in all the slaughterhouses, with some differences in specific stages. Basically, this process consists on a highly coordinated system of different operations aimed at slaughtering the birds, removing the inedible portions of the carcasses and preserving the edible portions for distribution to consumers (Sams and McKee 2010). The poultry slaughtering process involves the following phases: stunning and bleeding, scalding, defeathering, evisceration, washing and chilling. The whole process can be divided in two basic areas: the "dirty zone", including stunning, bleeding, scalding, defeathering and evisceration stages and the "clean zone" including washing and chilling (Escudero-Gilete et al., 2005).

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.

In the United States, E. coli was identified as a useful indicator organism to verify the adequacy of the hazard analysis and critical control points (HACCP) plans in place in bovine, swine and poultry slaughterhouses (USDA, 1996). In 1996, the FSIS

issued the Pathogen Reduction (PR)-HACCP System's Final Rule, prescribing that E. coli must be enumerated from 1/22000 and 1/3000 randomly collected broiler and turkey carcasses, respectively. The poultry carcasses will be selected after chilling and after the drip line, before packing/cut-up. A poultry establishment is considered to fulfil the E. coli process criteria if none out of the last 13 tests performed exceeds the upper limit of 1000 cfu/ml, and fewer than three samples are between 100 and 1000 cfu/ml for E. coli (Altekruse et al., 2009). These performance criteria for poultry allow microbial reduction during the slaughter processing to be monitored and interventions to reduce microbial numbers on poultry carcasses to be validated. Furthermore, poultry plants in the United States are required to meet the established *Salmonella* performance standard, consisting of a maximum of 12 *Salmonella*-positive samples in a complete set of 51 samples (Bilgili et al., 2010).

2.1.1: 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 are 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.

2.1.2: Scalding, Plucking and Evisceration: Scalding In this process turbulent hot water is used to transfer heat to the feather follicles, which then relax allowing feathers to be removed mechanically in the pluckers. Carcasses are conveyed through one or more scalding tanks filled with hot water at a preset temperature. The temperature of the scald water and the dwell time in the scalding system and therefore its size will depend on whether carcasses are to be soft (50±51ëC for 2.5±3 min), medium or hard scalded (58±60ëC for 1.5±2 min) for sale fresh or frozen. Soft scalding leaves the epidermis intact allowing soft scalded birds to be chilled by air alone. Medium and hard scalding will tend to loosen the outer layer of the epidermis, which is then partially removed during plucking. Such carcasses will usually have to be wet chilled and subsequently frozen to safeguard their appearance.G.C Mead, etal (2004)

Stals (1996) reported that most critical points, for cross-contamination during processing are scalding, plucking, and 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 bars) for the scald water reduces the number of Enterobactericeaes (Van and Mudder 1996).

Kim et al, (1993). Found 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 miroflora, 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 Rubin 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).

The water baths used during the process have a washing effect that diminishes the bacterial loads, but can also promote cross-contamination between carcasses. The high temperatures (50 to 60◦C) of the hot water used for scalding contribute to stopping bacterial growth. This helps to diminish the bacterial counts present on skin. However, high temperatures dilate feather follicles and relaxpoultryskin. Further processing steps may therefore lead to bacteria transferfrom feathers to skin and follicles, previously dilated by the hotwater and to entrapping bacteria after the cooling of plucked carcasses. Cold water used for chilling carcasses after evisceration can act as a cross-contamination vehicle between carcasses, but also has a decontaminating effect by rinsing the surface of carcasses , Arno Swart etal, (2015). 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 m 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.

Although cold water and air chilling procedures have different effects on diminishing Salmonella and Campylobacter counts, no difference has been observed in the impact of the two procedures on the shelf life of cuts The evisceration step, because of the microbiota present at high counts in the digestive tract, is a critical point of carcass contamination, Arno Swart etal, (2015).

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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 gastro-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$^{\circ}$ or 122F$^{\circ}$).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° to 60C° (138° to 140°F) (EncyclopediaBritannica, 1998).

2.1.3: 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 (EncyclopediaBritannica, 1998).

2.1.4: Chilling, Packaging, Storage:

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).

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 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 chicken's 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. There by preventing recontamination. Water chilling leads to an increase in poultry weight, and the amount of water gained is carefully regulated (EncyclopediaBritannica, 1998).

Although cold water and air chilling procedures have different effects on diminishing Salmonella and Campylobacter counts, no difference has been observed in the impact of the two procedures on the shelf life of cuts the evisceration step, because of the microbiota present at high counts in the digestive tract, is a critical point of carcass contamination.The effect of chilling carcasses using chilled air or a cold water-bath on their microbial contaminants has been assessed. Refrigeration by

chilled air slows down the development of the total viable count (approximately1log) and cause sarapid decrease in temperature. This inhibits the multiplication of *Salmonella and Campylobacter*, thus chilled-air cooling would be more efficient, Arno Swart etal, (2015).

Air chilling resulted in higher counts of *Enterobacteriaceae* on carcasses in one study (Barbut et al., 2009) and of *E. coli* in another one (Berrang et al., 2008a), but in contrast, no effects of chilling technique on E. coli levels on carcasses were observed in studies with chlorination (Sanchez et al., 2002; Barbut et al 2009) and without chlorination (Huezo et al., 2007). Chlorine was also judged as ineffective against indicator bacteria counts on carcasses compared to untreated water in immersion chilling (Russell and Axtell, 2005). However, these results are of limited utility in an EU perspective, because air chilling is widely applied, while the immersion technique is quite unusual. For these reasons, studies considering different renewal times for water used during immersion (Souza et al., 2012) are of limited value as are studies considering different ratios of water/Kg for processing (Northcutt et al., 2006; 2008c).

2.2: Bacteriology of poultry meat:

2.2.1: Microbiological status of broiler carcasses depends on several factors.

Suchas: 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 produces, 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 duringdefeathering, 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 flora 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, and Staphylococcus.*

2.2.2: Microbiological Quality of poultry Carcasses during slaughter house processing:

During the slaughter of poultry birds the contamination of carcasses, due to feed from gut of the birds which means bacteria present in spilled gut content is passed on as contaminants of importance is coliform especially *Escherichia Coli and Salmonella* . *Colibaccillosis*, *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).

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 (Klose, Kanfan and Pool 1971; Patrick, Colins, Goodwin 1973; Veekamp and Hofmans 1973.) .These minimize cross-contamination with *Salmonellas*.

Intestinal contents can be heavily infected with *Salmonellas* (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 salmonellas (Bryan et al 1968 ; Morris & Well , 1970) .

From a survey of contamination with Salmonella this 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, etal (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 & 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°), 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).

2.3.1: Bacterial Pathogens Associated with Poultry Meat:

Potential biological hazards in meat poultry includebacteria, 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 humanillness, 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;BrownandBairdParker.,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.The meat surface do not normally, inherently contain pathogenic organisms but can acquire the organisms from faecal matter or from cross contamination during slaughter. The organisms tend to remain on the surface or just under it. Meat is an ideal medium for bacterial growth because of high moisture content, richness in nitrogenous compounds (essential amino acids, proteins), good source of minerals, vitamins and other growth factors. Furthermore, its pH is favorable for the growth of micro-organisms. The water activity (aw) of poultry meat is about 0.98 to 0.99 depending on if and how long the meat has been stored in dry air. The pH of chicken breast muscle is 5.7 to 5.9, while that of leg muscle is 6.4 to 6.7. Both poultry muscle and skin are excellent substrates for supporting the growth of a wide variety of microorganisms (ICMSF, 2005).

Contamination of poultry carcasses and parts with *Salmonella* organisms is well documented and data are available for many parts of the world (Simmons et al., 2003). Most *salmonella* found on poultry meat are non-host-specific and are considered capable of causing human food poisoning. *Salmonellosis* (gastroenteritis) is the most common disease in human. Incubation period is generally 6 to 72 hours (Behravesh et al., 2008) and can be longer than 10 days. Symptoms include nausea, vomiting, diarrhea, abdominal cramps and fever of 100 to 102°F (Pickering, 2006)

2.3.2: The different bacteria were conducted in specific media (*Salmonella spp., Escherichia Coli and Staphylococcus aureus)*: -

2.3.2.1: *Salmonella*

The *Salmonellae* are gram-negative, non-spore-forming rod-shaped bacteria belonging to the family *Enterobacteriaceae*. However, *Salmonella* is not included

in the group of organisms referred to as coliforms. *Salmonella* is one of the principal causes of foodborne gastroenteritis worldwide and is also an important pathogen of livestock. Salmonellosis is a zoonotic infection (can be transmitted to humans from animals). *Salmonella* nomenclature has been revised over the years and is based on biochemical and serological characteristics. Many microbiologists now use a classification that recognises only two species of *Salmonella*. These are *S. enterica* (which includes 6 subspecies) and *S. bongori.* The subspecies most important in foodborne disease is *S. enterica* subspecies enterica. The genus *Salmonella* can be further divided into serotypes, of which there are a great many (42500). Most serotypes (sometimes referred to as serovars) be- long to the species *S. enterica* and only 20 belong to *S. bongori. Salmonella enterica* subspecies *enterica* contains nearly 1500 serotypes, including many of the serotypes that are known to cause foodborne disease. Under the currently accepted classification, an example of the correct way to denote a serotype would be *Salmonella enterica* subspecies *enterica* serotype Enteritidis, although fortunately convention allows this to be abbreviated to *Salmonella Enteritidis (S. Enteritidis).* In addition, each *Salmonella* serotype can be divided further by phage typing. A particular phage type can be denoted using the term PT. For example, *Salmonella Enteritidi sPT4* is an organism commonly associated with eggs and human illness. Other common serotypes involved in human illness are *S. Typhimurium and S. Virchow*. (Jay et al, 2003, Bell etal 2002, D, Aoust etal 2001)

2.3.2.1.1:Cultural characteristics:

Salmonella are aerobes and facultative anaerobes growing within a temperature range of 15 – 45c^{o} (optimum temperature 37c^{o}) they con grow on ordinary media.

2.3.2.1.2:Nutrient Agar Media or Blood Agar Media:

Colonies on these media are 2 - 3µm in diameter. Grayish – white, circular, moist, convex and translucent. Rough. (R)Strains form opaque and granular colonies, with irregular surface. They have hydrophobic surface andtend to auto agglutinate. Due to the production of losse polysaccharide slime, many strains of *S. paratyphi B*, and few of other serotypes from large mucoid colonies.

2.3.2.1.3:MacConkey Agar: Colonies are 1 - 3µm in diameter and pale yellow or colorless due to absence of lactose fermentation.

2.3.2.1.4:Billiant green MacConkey Agar: This is selective medium for isolation of *salmonella* from feces, *salmonella* produce green translucent colonies, however *S. typhi* dose not grow well on this media.

2.3.2.1.5:Dexoycholate Citrate Agar: Colonies are similar to or slight smaller in size than those of MacConkry Agar. After48 hour's incubation, the colonies may develop a black centre.

2.3.2.1.6:Wilson and Blair'sBrilliant–green BismuthSulphite Agar Medium:

On this medium *S.Typhi* and *S.baraTyphi* from small (about 1µm in diameter) black colonies this due to reduction of hydrogen sulphide.The colonies of *S. Typhi*. Are surrounded by metallic sheen Brilliant green inhibits. The growth of *E.coli*, Proteus and other commensally entrobactera.

2.3.2.1.7:Xylose Lysine Dexoycholate Agar: Most strains of *salmonella* produce red colonies with black centre.H_2S negative serotypes of *salmonella* produce red colonies without black centers.

2.3.2.1.8:*Salmonella – Shigella* Agar: Colonies of salmonella are color less with black centre

2.3.2.1.9:Hektoen enteric Agar: Colonies of *salmonella* are blue green with black centre due toH_2S production.

2.3.2.1.10:Enrichment media1/Tetrathionate broth: It enriches *Salmonellae* and sometimes *Shigellae*, but permits the growth of Proteus.

2.3.2.1.1.11/Brilliant green tetrathionate broth: Brilliant green in tetrathionate broth the growth of Proteus these make it more selective for the *Salmonellae*. But it is also inhibitory. To some extent, to *S.Typhi and Shigella*.

2.3.2.1.12/Selenite F. broth: It is an excellent enrichment medium for isolation of *S.Typhi and S, Dublin*, but some *Salmonellae e.g. S.paraTyhi A* and S.*Choleraesuis* and some *Shigellae* may fail to grow in this medium.

2.3.2.1.1.13: Hazard Characterization

Effects on Health

Some *Salmonella* serotypes have a limited host spectrum (i.e. they cause specific and often serious clinical disease in one or a few animal species), such as *S. Typhi and S. Paratyphi* in humans (causing typhoid fever), *S. Dublin* in cattle, and *S. Choleraesuis* in pigs. These are not considered further here. The more usual foodborne form of the illness is caused by *non-typhoid salmonellae*, which invade the cells lining the small intestine. These organisms cause gastroenteritis lasting between 1–7 days, with symptoms that include diarrhoea, abdominal pains, nausea, vomiting, and chills, leading to dehydra- tion and headaches. Susceptible individuals, such as the young, the elderly and those who are immunocompromised can sometimes develop more severe symptoms from *non-typhoid salmonellae* such as septicaemia, or chronic conditions, such as reactive arthritis. The death rate for infection by non- *typhoid salmonellosis* is10% although this figure is higher amongst some groups, particularly the elderly. The incubation time is between 6 and 48 (usually 12–36) h. The infective dose is thought to vary widely and can depend on the individual consuming the in- fected food, the type of food involved and possibly the serotype involved. Small numbers (between 10–100) of cells can cause illness if

consumed by the young or the elderly, or if the food consumed has a high fat content (e.g. chocolate, cheese or peanut butter) because the fat is thought to protect the cells from the gastric acids. In general however, it is thought that high numbers (between 105–106 cells) of *salmonellae* need to be consumed to cause illness. Individuals recovering from *salmonellosis* can continue to shed *Salmonella* in their stools for some time. Food handlers reporting *Salmonella* gastroenteritis should be excluded from work until shedding has stopped. (Jay et al, 2003, Bell etal 2002, D, Aoust etal 2001).

2.3.2.1.1.14: Salmonella and Paracolon Infections

There are more than 2,000 species or serotypes of bacteria belonging to genus *Salmonella*; all are potential pathogens of poultry. Systemic effects usually are observed when infection occurs, but because the digestive system is primarily affected, they often are referred to as enteric organisms. The same is true of the group of organisms referred to as par colons. Because of similarities produced by infections by these organ- isms, they are grouped under one heading. Both groups are found worldwide. Pullorum disease and fowl typhoid are infectious, acute, or chronic bacterial diseases affecting primarily chickens and turkeys, but most domestic and wild fowl can be infected. The causes are bacteria, *Salmonella pullorum and S. gallinarum*, respectively. Transmission is primarily through the egg but may occur by other means such as:

1. Infected hen — egg — infected chick — spread in incuba- tor — in chick boxes — in brooder house and on range — survivors become infected breeder birds.
2. Mechanical transmission — carried about on shoes or equipment.
3. Carrier birds — apparently healthy birds which shed organisms.
4. Contaminated premises — from previous outbreaks.

Portal of entry may be the respiratory (as in incubator) or digestive system. Signs: Pullorum disease is highly fatal to young chicks or poults, but mature birds are more resistant. Young birds may die so soon after hatching that no signs are observed. Most acute outbreaks occur in birds under 3 weeks of age. Mortal- ity in such outbreaks may approach 90 percent if untreated. Survivors usually are stunted or unthrifty.

Infection in young birds may be indicated by droopiness, ruffled feathers; a chilled appearance with birds huddled around the source of heat, white diarrhea with "pasted" down around the vent, and labored breathing. Fowl typhoid primarily occurs in young adults (usually those past 12 weeks of age). Signs include sudden or sporadic mortality, listless-ness, green or yellow diarrhea with pasting of the vent feathers, loss of appetite, increased thirst, and a pale, anemic appearance of comb and wattles. Irena (2012)

2.3.2.1.3: Diagnosis:

The diagnosis is made by isolating the causative organism. In older birds, blood testing may indicate presence of the disease, but a positive diagnosis depends upon isolation and identification of the organism by laboratory methods. Prevention: Complete eradication is the only sound way to prevent pullorum disease. All hatchery supply flocks should be tested and only pullorum-free flocks used to produce hatching eggs. Producers should always purchase chicks or poults from hatcheries that participate in the National Poultry Improvement Plan, which includes an official pullorum disease control program.

Treatment: Treatment is primarily a salvage operation and does not prevent birds from becoming carriers. Consequently, recovered flocks should not be kept for egg production.

Paratyphoid: first was used to designate a group of human, feverish conditions resembling typhoid fever. Related to poultry, paratyphoid denotes the disease produced by any of the many *Salmonella species other than S. pullorum and S. gallinarum*. Infection may result in acute or chronic disease. Acute clinical disease is common in young birds and rare in adult birds. Over 2,000 species or serotypes of *Salmonella*organisms are recognized, and most birds, reptiles, and mammals can host one or more species. The disease is of greatest economic concern to the turkey industry. Most acute paratyphoid infections occur in birds less than 4 weeks old, except in pigeons and canaries in which acute disease and high mortality may occur in any age group.

Bacterial Diseases of Poultry Excluding Respiratory Diseases

(Oklahoma Cooperative Extension Fact Sheets**)**

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 fasces 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 fesces (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 grows 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.

2.3.2.1.4: Legislation

There are codes of practice in many countries around the world for the pro- duction of various food commodities that include measures to control *Salmo- nella*. Although it is unacceptable for any ready-to-eat product to contain viable

Salmonellae, there are regulations in many countries enforcing requirements in specified products. European Union regulations have specific requirements pertaining to *Sal- monella* in a wide range of products, including meat and meat products, cheese, butter and cream that have not undergone standard pasteurisation processes, milk powder, whey powder, some ice cream and egg products, various shellfish products, ready-to-eat sprouted seeds, ready-to-eat fruit and vegetables, un-pasteurised fruit and vegetable juices and infant formula and dried dietary foods. Sampling plans and absence requirements vary depending on product. There are also EU requirements for *Salmonella* testing of cattle, sheep, goats, horses, poultry and pig carcasses.

US food law also requires *Salmonella* to be absent from ready-to-eat food products that are not intended to be heated before being consumed. There are also specific requirements for the labeling of eggs not treated to inactivate the pathogen and for control of *Salmonella* in foods prepared for vulnerable populations. Some countries have specific storage, labeling requirements and heat treat- ments for foods that are aimed at controlling foodborne *salmonellosis*. In the US these include mandatory refrigerated storage of eggs (from farm to the consumer) and labeling requirements for the inside of egg boxes advising of safe egg-handling practices. In the EU, legislation requires many eggs to be stamped with a distinguishing mark and country of origin to help trace the farm of origin in case of an outbreak. Richard Lawley, etal (2008).

2.3.2.2: *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 (endodermis) (Q. Ashoton action 2o13) 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 has 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_2, and by preventing the establishment of pathogenic bacteria within the intestine (Bentley and Meganathan, 1982).

E. coli and Enterobacteriaceae counts on poultry carcasses depend on the plant where the slaughtering process took place. The effect of the size of the slaughterhouse on the indicator bacteria counts was investigated in several papers (Sumner et al., 2004; Lindblad et al., 2006, Bohaychuk et al., 2009). Summer et al. (2004) provided a description of slaughterhouses according to their main technical features, whereas in the latter two papers, specific descriptions of the slaughtering practices were not provided. It was concluded that slaughterhouses play an important role in the indicator bacteria counts on carcasses; however, it is not possible to understand what effect the practices applied at the different stages of the slaughter processing line have on indicator bacteria counts. Bohaychuk et al. (2009), dealing with this point, concluded that the high volume abattoir had lower counts if the results of rinse analysis were expressed as CFU/ml, but had higher counts if the results were transformed into CFU/cm2. This finding should be carefully considered when evaluating results obtained with the rinse sampling technique. Sumner et al.

(2004) concluded that the dimension of the slaughterhouse had no effect on indicator bacteria counts (expressed on a cm2 basis), because the lowest counts were found both in the largest and the smallest abattoirs. No effect was observed also in relation to the level of mechanization.

2.3.2.2.1:Cultural characteristics: It is an aerobe and facultative anaerobe optimum temperature for its growth is 37c^{o} range (10-45c^{o}), it can grow on ordinary media like Nutrient.

2.3.2.2.2:Nutrient Agar Media: Colonies are large (2 – 3mm in diameter), circular, convex, colourless, opaque or partially translucent, after 18hours incubation 37c^{o}

2.3.2.2.3:MaConkey Agar: Colonies are red or pink in color due to lactose fermentation,

2.3.2.2.4:DexyoCholate Citrate Agar (DCA) The growth is partially or totally inhibited by sodium citrate and sodium thiosulphate, colonies of some strains of *E.coli* are surrounded by complete zone of haemolysis.

2.3.2.2.5: Hazard Identification

VEROCYTOTOXIN-PRODUCING ESCHERICHIA COLI (VTEC)

The verocytotoxin-producing *Escherichia coli* (VTEC) are a group of strains within the species *E. coli*, some of which are highly pathogenic and capable of causing potentially serious foodborne infections in humans. *E. coli* are gram- negative, non-spore-forming bacteria belonging to the family *Enterobacteriaceae*. Microbiologists recognise a small number of genera within the *Enterobacteri- aceae,* including *Escherichia* species, as the coliform group. *E. coli* are found as part of the normal human gut flora, as well as in the environment, and the presence of *E. coli* in processed product can indicate faecal contamination (the reason why *E. coli* is used as an ‘‘indicator’’ organism). Most strains of *E. coli* do not usually cause illness, but a minority has been associated with infections resulting in diarrhoea, or sometimes

more severe illness. There are four different groups of diarrhoea-causing *E. coli* grouped by virulence characteristics as follows:

Enteropathogenic (EPEC) causing infantile gastroenteritis or summer diarrhoea mostly in the developing world.

Enterotoxigenic (ETEC) causing traveller's diarrhoea

Enteroinvasive (EIEC) causing a form of bacillary dysentery

Verocytotoxin-producing (VTEC) – some- times referred to as Shiga-like toxin-producing (STEC). This group includes a subset of serotypes often referred to as enterohaemorrhagic *E. coli* (EHEC)

Not all VTEC are associated with human disease, but those that are EHEC can cause haemorrhagic colitis (bloody diarrhoea).

The group of most concern in developed countries is the VTEC, so named because they produce one or more toxins that are toxic to vero cells (a tissue cell culture line derived from the kidneys of an African Green monkey). In excess of 200 VTEC have been described and some of these organisms have been asso- ciated with outbreaks of severe foodborne disease in many countries. The VTEC most frequently associated with causing foodborne illness is the serotype *Escherichia coli O157:H7*. Other important VTEC that have caused foodborne infections are O26, O103, O111 and O145.Desmarchelier, etal (2003).

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). It^{s} 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; et al, Adetunji, 2011), properly due to increase used antimicrobials (Miranda et al, 2008).*Coliform* Infections, *Colibacillosis Coli* forminfections refer

to the many and various disease resuling from infection with *Escherichia coli* bacteria. In recent years these infections have become recognized as a major cause of morbidity, mortality, and condemnations in chickens and turkeys. The incidence and severity of *coliform* infections have increased rapidly, and current trends indicate they are likely to become an even bigger problem. The problems attributed to *coliform* infections are often complex. There is a marked variation in severity. Problems range from severe acute infections with sudden and high mortality to mild infections of a chronic nature with low morbidity and mortality. Infections may result in a respiratory disease from air sac infection, a septicemic disease from generalized infection, enteritis from intestinal infection, or a combination of any or all of these. Disease may result from *coliform* infection alone as in primary infection or in combination with other disease agents as complicating or secondary infection. Secondary infections commonly occur as a part of the classic air sac disease syndrome as a complication of *Mycoplasma gallisepticum* infections. All ages may be affected; however, it is more common in young growing birds, especially the acute septicemia in young turkeys and air sacculitis in young chickens. High early mortal- ity may occur as the result of omphalitis or navel infections. Cause: The disease is caused by *E. coli* bacteria and from toxins they produce as they grow and multiply. There are many different strains or serological types within the group of *E. coli* bacteria. Many are considered normal inhabitants of the intestinal tract of chickens and turkeys and consequently are common organisms in the birds' environment.

The primary routes of invasion by the organism are the respiratory system and the gastrointestinal tract. Omphalitis and infections in young birds may result from entry of the organism through the unhealed navel or penetration of the egg shell prior to or during incubation. Symptoms: The symptoms vary with the different types of infections. In the acute septicemic form, mortality may begin suddenly and progress rapidly. Morbidity may not be apparent and birds in apparently good condition may die. However, in most cases, morbid birds are evident as listless birds with ruffled feathers and indications of fever. In the chronic infection, debilitation, and growth retardation are obvious. In the event of respiratory infection, additional symptoms of labored breathing, occasional coughing, and rales may be apparent. In the case of enteritis, diarrhea may be evident. Mortality may be high in recently hatched chicks and poults as a result of omphalitis due to coli form infections.

2.3.2.2.6: Diagnosis:

Differential diagnosis by laboratory means is necessary since *coliform* infection in its various forms may resemble and be easily confused with many other diseases. Isolation and identification of the organism by culture procedures can be readily accomplished; however, mere isolation is not sufficient to make a diagnosis. One must take into consideration the organ from which the organisms were isolated, the pathogenicity of the particular isolate and the presence of other disease agents.

2.3.2.2.7: Prevention:

Management and sanitation practices designed to minimize the exposure level of these types of organisms in the birds' environment are necessary in any preventive program. In addition, these programs should include avoiding stress factors and other disease agents which may lower the resistance and predispose the birds to infection. Important points in these management and sanitation practices include providing adequate ventilation, good litter and range conditions, properly cleaned and disinfected equipment and facilities, and feed and water supply free of contamination. In addition, these programs should include avoiding overcrowding and environmental stresses such as chilling and overheating, and avoiding vaccinating and handling at critical times. Proper egg handling, as well as a good hatchery management and sanitation program, are necessary to prevent early exposure.

2.3.2.2.8: Legislation

EU regulations have some general requirements for *E. coli* as an indicator of faecal contamination in some products. These requirements giving maximum levels for *E. coli* in some products do not pertain specifically to VTEC, but the presence of VTEC in any product that will not receive a heat treatment prior to consumption is unacceptable. The UK Health Protection Agency has issued guidelines for the microbiological quality of ready-to-eat foods and these state that in these products *E. coli O157* and other VTEC should be absent in 25g. The US Food Code (2005) requires food to be safe and unadulterated and product that will not be heated prior to being consumed would need to be absent from VTEC to conform to this requirement. In addition, *E. coli O157:H7* is considered an adulterant in non-intact raw-beef products (ground, minced or chopped), as well as intact raw-beef products

intended to be pro- cessed into non-intact raw-beef products. Richard Lawley, etal (2008).

2.3.2.3: Staphylococci Spp.:

Staphylococci are facultative anaerobic, catalase-positive, oxidase-negative, non-motile, non- spore forming and fermentative bacteria. Colonies appear smooth, raised, glistening, circular, and entire. Single colonies can attain a size of 4-6 mm in diameter on non-selective media. Colony color is variable, from grey or grey-white to orange (Tsegmed, 2006). They are Gram- positive bacteria, with diameters of 0.5 – 1.5 μm and characterized by individual cocci, which divide in more than one plane to form grape-like clusters Harris et al., (2002). *Staphylococci* are ubiquitous in the environment. Natural populations are associated with skin, skin glands and mucous membranes of warm-blooded animals. They have been isolated from animal products such as meat, milk and cheese, and other sources such as soil, sand, seawater, fresh water, dust and air Harris et al., (2002). Some *Staphylococcus*species are known to be frequently encountered in severe infections. Historically, only the *Staphylococcus aureus* was considered to be pathogenic. *Staphylococcus aureus,* a species which produces a variety of enzymes and toxins, is the best known and frequently implicated in the etiology of a series of infections and intoxications in animals and humans, whereas coagulase-negative *Staphylococcus* (CNS), representing the majority of species, have been considered to be saprophytic or rarely pathogenic Cuncha et al., (2004). In the last two decades coagulase-negative *Staphylococcus* species have emerged as significant pathogens, especially in medical-device-related infections and in immune-compromised patients Cuncha et al., (2004).

Food-borne diseases are common in developing countries including Ethiopia because of the prevailing poor food handling and sanitation practices, inadequate

food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipments, and lack of education for food-handlers WHO, (2004). Report highlighted that foods prepared in large quantities are liable to contamination (WHO, 2005). Prepared foods should also get proper handling and preservation in order to safeguard public health. Abattoirs are one of the food industries that contribute to the problem of possible food-borne diseases and potential health hazards associated with food unless the principles of food hygiene are implemented. Meat handlers are probable sources of contamination with microorganisms; it is important that all possible measures are taken to reduce or eliminate such contamination Haileselassieetal, (2012). Food-borne pathogens are one of the leading causes of illness and death in developing countries costing billions of dollars in medical care and social costs Fratamico et al, (2005). Changes in eating habits, mass catering complex and lengthy food supply procedures with increased international movement are major contributing factors Iroha et al, (2011). Contaminated raw meat is one of the main sources of food-borne illness Bhandare et al., (2007); Podpecan et al, (2007). Bacterial contamination of meat products is an unavoidable consequence of meat processing. Even if data regarding meat borne diseases in Ethiopia are extremely scarce, a few studies conducted in different parts of the country have shown the public health importance of several bacterial pathogens associated with foods of animal origin Haileselassie et al, (2012).

Common bacterial contaminants of meat samples are *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella species, Enterococcus species, Enterobacter species and Citrobacter species.* The higher rate of contamination of meat with these organisms is an indication of unacceptable state of poor hygienic and sanitary practices employed right from the slaughtering, transportation, butcher shops and processing Haileselassie et al, (2012).

Bacteriological examination of meat in Mekelle city municipal abattoir of Ethiopia indicated the predominant presence of *Staphylococcus aureus, Escherichia coli and Bacillus cereus* Haileselassie et al., (2012).

Staphylococcus aureus is always considered to be potentially pathogenic and responsible for many human diseases. The clinical syndromes caused by this bacterium can be coutaneous infections, which includes folliculitis, impetigo, wound infections, toxin-mediated infections that include toxic shock syndrome, food poisoning, scalded skin syndrome which is seen in children under the age of four, other diseases such as pneumonia, bacteremia, endocarditis, osteomyelitis and septic arthritis Sila, (2006).

The occurrence of *Campylobacter species, Staphylococcus species, Escherichia coli, Salmonella species, Yesinia species and Listeria* species in meat, sea foods, vegetable ingredients, raw and cooked foods, raw chicken, beef burger sandwiches, ready-to eat salad vegetables, commercial mayonnaise, poultry products and on the hands of food workers was reported at Al-Taif Governorate, Kingdom of Saudi Arabia (Eman and Sherifa, 2012). According to Mohammad et al. (2010), bacterial counts exceeding 105/g in ready to eat food products are indicative of dangerous contamination in Tando Jam, Pakistan. Numerous *staphylococcal* enterotoxins have been described and it is ingestion of these enterotoxins and not of *Staphylococcus aureus* cells that causes a rapid onset of nausea and vomiting within 1–6 hours (Doyle et al., 2011).

*2.3.2.3*Diseases Caused by *Staphylococci:*

Staphylococci are human pathogen, known for their ability to become resistant to antibiotics. *Staphylococci* cause different types of infections or diseases in a host such as endophthalmitis, osteomyelitis, endocarditis, chronic skin infections,

indwelling medical device infections, chronic rhino-sinusitis, abscess, sepsis, dental implantits and others Choudhury et al., (2011).

Coagulase negative and positive *staphylococci* are responsible for a variety of anterior and posterior segment of eye infections such as blepharitis, canaliculitis, dacryocystitis,

Conjunctivitis, keratitis, scleritis, endophthalmitis, preseptal and orbital cellulitis etc Choudhury et al., (2011).

2.3.2.3.1: *Staphylococcus aureus*

The growth of *Staphylococcus aureus* in foods is a potential public safety hazard since many of its strains produce enterotoxins that cause food poisoning when ingested Humberto, (2004). *Staphylococcus aureus* most frequently causes diseases in humans in various suppurative (pus- forming) infections. It causes superficial skin lesions as boils and furunculosis, more serious infections such as pneumonia, mastitis, and urinary tract infections, and deep-seated infections such as osteomyelitis and endocarditis (Ellis et al., 2003; Tsegmed, 2006). *Staphylococcus aureus* is one of the most economically important bacteria responsible for the cause of food borne poisoning worldwide (Bennett, 2005; Tsegmed, 2006).

In animals, *Staphylococcus aureus* can cause pustular inflammation of the skin and other organs. *Staphylococcus aureus* is an important cause of mastitis in cattle, sheep and goats (Yazdankhah et al., 2001; Rodrigues da Silva et al., 2005).

.3.2.3.1.1:Cultural characteristics:

They are aerobes and facultative anaerobes, optimum temperature for growth is 37c^{o}, range being 12 -44 c^{o} optimum. PH is 7.5; they can grow well on ordinary media.

.3.2.3.1.2:Nutrient Agar: After overnight incubation 37c^{o}, colonies are 1 -2 µm in diameter with smooth glistening surface, they are opaque and easily emulsifiable, most strains, most strains produce golden – yellow (aureus), pigment, though some strains may from white colonies

.3.2.3.1.3:MacConekey: Colonies are smaller and pink color due to lactose fermention.

.3.2.3.1.4:Blood Agar: Colonies are similar to that Nutrient Agar but may be surrounded by zone of β haemolysis.

.3.2.3.1.5:Mannitol Salt Agar: This is selective and indictor medium. Colonies are similar to those Nutrient Agar but they are surrounded by yellow zones due to fermentation of mannitol by most strains of S.aureus.

.3.2.3.1.6:Phenolphthalein phosphate **Agar:** This is an indicator medium, this assist in the identification of S. aureus in mixed culture colonies become bright pink in minute because phenolphthalein is pink in alkaline PH.

2.3.2.3.2: Legislation

EU legislation has requirements governing sampling plans and limits for coa- gulase-positive staphylococci in various cheeses, milk powder and whey powder. For these foods levels of coagulase positive staphylococci below 10–104 CFU/g (depending on product) at the time of removal from the premises are generally satisfactory. However, tests for staphylococcal enterotoxin are required where levels of coagulase-positive staphylococci are detected at 4105 CFU/g, and these toxins should be absent in 25g. If coagulase-positive staphylococci are found at levels 4103 CFU/g in shelled and shucked products of cooked crust- aceans and molluscan shellfish, EU regulations require improvements in pro- duction hygiene. The US

Food & Drug Administration's (FDA) food compliance program suggests that any cheese or fish product could be removed from the market place if it is found positive for staphylococcal enterotoxin or if levels of Staphylococcus aureus are Z104 CFU/g. The UK Health Protection Agency (HPA) has issued guidelines on the microbiological quality of some ready-to-eat foods at the point of sale. These state that levels of Staphylococcus aureus of 100/g to o104/g in these products is unsatis-factory, and levels 4104/g is unacceptable/potentially hazardous. Richard Lawley, etal (2008).

2.4: FOOD SAFETY

Food safety can be defined as the system that keeps food and food products free from substances hazardous to human health. Food safety should be a part of governments' strategies to ensure secure food for the consumers. In this context, a "hazard" refers to any biological, chemical or physical property that may cause unacceptable risk (FAO, 1998). The emergence and discovery of new food-borne pathogens and other food-related hazards has increased the need for food-safety measures. The intensification of food production has also changed food processing and handling systems and raised new challenges for food- safety institutions. Intensification has led to large amounts of potentially infectious material being concentrated at single sites, such as large industrial production establishments or processing plants, and has therefore contributed to the potential for large-scale outbreaks of infection. Changing consumption patterns – street vendors and home cooking of primary products are giving way to the purchase of processed food from supermarkets – make food-safety an issue of public concern rather than just a matter for individual consumers. Developing countries face difficulties in achieving food-safety goals in animal production systems. These difficulties result from inter alia unstable administrative and political structures, lack of infrastructure, and lack of investment in food-safety measures and research, as well as from inadequate consumer information. Responsibility for ensuring safe food for the consumer has traditionally been seen as the responsibility of public institutions. However, with the

intensification and industrialization, responsibility has been shifted to a wider set of stakeholders including the private producer and the consumer.

2.4.1: The potential Risk Factors:

Three types of food-borne risk factors for human health can be recognized (FAO, 1998). The first group of risk factors comprises microbiological factors such as *Campylobacter spp. and Salmonella spp*. The second group of risk factors comprises chemical factors such as residues from veterinary medications, pesticides, natural toxins or environmental pollution. Excessive use of medication during poultry production, or disinfectants used in the food- processing industry, can give rise to the problem of resistance. This adds to the problem of food hygiene. The third group of risk factors comprises physical hazards such as bone- pieces in meat .

It is suggested that the first step, pre-harvest control, is the most

important means to prevent infection with pathogens such as *Salmonella*, as traditional control systems are unable to control for these pathogens later in the chain. Singer et al, (2007) describe three reasons why it is important to process only healthy animals – thus emphasizing the importance of pre-harvest measures. First, a sick animal will shed pathogens into the surroundings and onto other animals; second, processing a sick animal may require additional handling in order to separate the infected parts from the carcass, which may add to the risk of cross-contamination; and third, certain illnesses lead to pathological changes in the carcass which may cause increased fragility of specific organs. *E. coli*-originated airsacculitis, which causes adhesions of the inner organs and therefore increased risk of ruptures during mechanical processing and increased risk of cross-contamination.

Slaughterhouses and food-processing establishments are the next links in the chain of food safety. The post-slaughter poultry carcass is a suitable growing medium for many pathogens, including human pathogens. Hygiene procedures when handling the carcass are, therefore, crucial and should be carefully planned and monitored to avoid contamination and cross-contamination of the food products. Packaging, transport, shelf-life and storage, as well as the maintenance of the cold-chain are important considerations. The cleaning and disinfecting of the premises and transport vehicles involved in these processes should be controlled. Resistance issues should be considered in the choice of the products used. Food products are

then transported to wholesalers, retailers and finally to the consumers. Many cases of food-borne illnesses could be avoided by applying good hygiene practices in the home or in restaurants. Consumer information and education is, therefore, crucial, especially in developing countries where hygiene standards are poor.

Responsibilities for control three major stakeholders can be identified in an industrial poultry production chain– the producer, the consumer, and the government. In industrialized countries, there are strong consumer-protection organizations which directly, or indirectly through governmental institutions, put pressure on the producer to supply safe products. A shift of legal responsibility from the government to the producer has been the common trend in developed countries FAO, (2007). According to this mindset, the optimal role of the government is as a guarantor of the system through administrative and regulatory methods – the producer being the one managing the systems. A major factor in the prevention of food-borne illnesses is to ensure that stakeholders from all sides understand their responsibilities and voluntarily introduce good hygiene practices

The check points at which contamination usually assessed belong to Hazard Analysis and Control of Critial Points (HACCP).

2.4.2: Hazard Analysis and Control of Critial Ponits (HACCP):

2.4.2.1: 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.

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.

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 to 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. GMP regulations are designed to control the risk of contaminating foods with filth, chemicals, microbes, and other means during their manufacture.

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

2.4.2.2: Seven Principles of HACCP:

The following seven principles of HACCP were adopted by the National Advisory Committee on Microbiological Criteria of Foods (NACMCF, 1992).

1/ Conduct a hazard analysis. Prepare a list of steps in the process where significant hazards occur and describe the preventive measures.

2/ Identify the critical control points (CCPs) in the process. A critical control point is defined as a point, step or procedure at which control can be applied and a food safety hazard can be prevented, eliminated or reduced to an acceptable level.

3/ Establish critical limits for preventive measures associated with each identified CCP. A critical limit is defined as a criterion that must be met for each preventive measure associated with a CCP. Each CCP will have one or more preventive measures that must be properly controlled to assure prevention, elimination, or reduction of hazards to acceptable levels. Each preventive measure has associated with it critical limits that serve as boundaries of safety for each CCP.

4/ Establish CCP monitoring requirements. Establish procedures for using the results of monitoring to adjust the process and maintain control.

5/ Establish corrective action(s) to be taken when monitoring indicates that there is a deviation from an established critical limit.

6/ Establish effective record-keeping procedures that document the HACCP system.

7/ Establish procedures for verification that the HACCP system is working correctly

2.4.2.3: 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.

4/ Product Flow. In the product flow were included 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 Quality. Several parameters have been discussed to ensure a safe product.

6/ Critical limits selected must be based on the best information available to provide a safe product.

Processors must keep in critical limit must have a Corrective Action taken on the product before being released from the plant.

Process Authority Reference 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: 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. Refers to the point at which a plant gains control of the meat. The history of meat products.

10/ Sampling Procedures. Establish a sampling plan to verify critical control points (biological, chemical and physical) in the operation..

Poultry Slaughter Model

USING THIS GENERIC MODEL TO DEVELOP AND IMPLEMENT A HACCP PROGRAM

Getting Started: The plant should establish a HACCP team which includes at least one HACCP trained individual, and then develop a flow chart for each product. In addition, a training program should be completed for all employees. It is important for all employees to have ownership in the HACCP plan and to participate in its development as appropriate. It also is important that the employees be given the authority to stop production if the process becomes out of control. This empowerment is critical to make the HACCP program a successful one. Once HACCP is established, it must be continually evaluated, upgraded, and modified. Experience in working a HACCP plan will be helpful in continual improvement in the plan. In effect, the HACCP program is a long-term commitment to improving the safety of the product by controlling the process.

Chicken Processing

Unloading/Hanging/Stunnin/Killing/BleedingScalding/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

Analysis and identify steps in the process where significant hazards can occur. The significant hazards must be "of such a nature that their prevention, elimination, reduction or control to acceptable levels is essential to the production of safe food." (NACMCF, 1992) The team should focus on risk and severity as criteria for determining whether a hazard is significant or not. Risk, as defined by the National Advisory Committee, is "likelihood of occurrence." "The estimate of risk is usually based on a combination of experience, epidemiological data, and information in the technical literature." (NACMCF, 1992). Severity is the potential magnitude of the consequences to the consumer if the hazard is not adequately controlled. Hazards that are not significant or not likely to occur will not require further consideration in the HACCP plan.

Hazard Analysis Critical Control Point (HACCP) Systems regulation (USDA, 1996).

The NACMCF has 12 steps (five preliminary steps listed below and the seven principles previously listed) in developing a HACCP plan.

2.4.2.4: HACCP Plan

PRELIMINARY STEP

1 (:Assemble the HACCP team.

2) Describe the food and its method of distribution.

3) Identify the intended use and consumers of the food.

4) Develop a flow diagram which describes the process.

5) Verify the flow diagram.

Then apply the seven principles beginning with conducting a hazard analysis.

The following steps should be considered when developing an effective HACCP system.

Before developing the HACCP system it is important to ensure that an adequate sanitation system (sanitation standard operating procedures - SSOPs) is in place for compliance with FSIS regulation. GMPs and SOPs are also important because they establish basic operational parameters for the production of safe food.

2.4.2.4.1: Assembling the HACCP Team: An important step in developing a plan is to gain management commitment and assemble a HACCP team. Top management must be fully committed to product safety through HACCP to make the program effective. After commitment is obtained, the HACCP team should be assembled. The team should consist of individual(s) from all aspects of production and should include at least one HACCP trained individual.

2.4.2.4.2: Product Description. The description should include the products within the process, their distribution, intended use, and potential consumers. This step will

help ensure that all areas of concern are addressed. If a particular area on the example form is not applicable to your process, then eliminate it from your description. The description for the Poultry Slaughter is included in this model.

2.4.2.4.3: Flow Diagram. The HACCP team should develop and verify a flow diagram for production of the product(s). A simple flow diagram which includes every step of production is necessary. The flow diagram should be verified for accuracy and completeness by physically walking through each step in the diagram on the plant floor. The purpose of the flow diagram is to provide a clear, simple description of the steps in the process which are directly under the control of the facility. This model contains a generic flow diagram for Poultry Slaughter.

2.4.2.4.4: Hazard Analysis. A hazard has been defined as any biological (B), chemical (C) or physical (P) property that may cause a food to be unsafe for human consumption. The hazard analysis is one of the most critical steps in the development of a HACCP plan. The HACCP team must conduct a hazard.

2.4.2.5: Conception of Hazard Analysis and Control of Critical Poimts (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).

2.4.2.5.1: Biological Hazards :

The following biological hazards should be considered:

Pathogenic microorganisms:

Bacillus cereus /Campylobacter jejuni /Clostridium botulinum/Clostridium perfringens /Escherichia coli O157:H7 /*Listeria monocytogenes/ Salmonella spp/ Staphylococcus aureus /Yersinia enterocolitica*

Zoonotic agents: Trichinella spiralis /Taenia saginata /Taenia solium/Toxoplasma gondii /Balantidium coli /Cryptosporidium spp.

2.4.2.5.2: Chemical Hazards :

The following sources were identified.

1) Agriculture chemicals: pesticides, herbicides, animal drugs, fertilizers, etc.

2) Plant chemicals: cleaners, sanitizers, oils, lubricants, paints, pesticides, etc.

3) Naturally-occurring toxicants: products of plant, animal or microbial metabolism such as aflatoxins, etc.

4) Food chemicals: preservatives, acids, food additives, sulfating agents, processing aids, etc.

5) Environmental contaminants:

Lead, cadmium, mercury, arsenic, PCBs

2.4.2.5.2: Physical Hazards:

Glass, metal, stones, plastics, bone, bullet/BB shots/needles, jewelry, etc. The hazard analysis and identification of associated preventive measures accomplishes the following: Identifies hazards of significance and associated preventive measures. The analysis can be used to modify a process or product to further assure or improve food safety. The analysis provides a basis for determining CCPs, principle 2.

2.4.2.5: Critical Control Point (CCP): A CCP is any point, step, or procedure at which control can be applied so that a food safety hazard can be prevented, eliminated, reduced, or controlled to acceptable levels. Information developed during the hazard analysis should enable the HACCP team to identify which steps in the process are CCPs. A decision tree, such as the NACMCF Decision Tree may be useful in determining if a particular step is a CCP for an identified hazard.

The CCPs discussed in this generic model should be considered as examples. Different facilities preparing the same product can differ in the risk of hazards and the points, steps, or procedures which are considered CCPs. This can be due to differences in each facility layout, equipment, selection of ingredients, or the production process that is being used. Plant-specific HACCP plans may include additional or fewer CCPs than this model based on their individual process.

Critical Limit: A critical limit is a criterion that must be met for each preventive measure associated with a CCP. Therefore, there is a direct relationship between the

CCP and its critical limits that serve as boundaries of safety. Critical limits may be derived from sources such as regulatory standards and guidelines, scientific literature, experimental studies, and advice from experts. The HACCP worksheet provided in this model summarizes the critical limits for each CCP. Critical limits must be based on the best information available at the time to provide a safe product and yet must be realistic and attainable. Establishments must keep in mind that any product which does not meet the critical limit must have a Corrective Action taken. Corrective actions may be as simple as re-processing or re- packaging or may require destroying the product.

2.4.2.6: Monitoring:

Monitoring is a planned sequence of observations or measurements to assess whether a CCP is under control and produces an accurate record for future use in verification. Monitoring serves three purposes: 1) Monitoring is essential to food safety management in that it tracks the systems operation. 2) Monitoring is used to determine when there is a loss of control and a deviation occurs at a CCP, exceeding the critical limit. Corrective action must then be taken. 3) Monitoring provides written documentation for use in verifying the HACCP plan.

Because of the potential serious consequences of a critical defect, monitoring procedures must be effective. Continuous monitoring is possible with many types of equipment, and it should be used when possible SSOP and GMP Programs for Poultry Abattoir:

Poultry meat processor must be understanding the basic principles behind Sanitation Standard Operating Procedures (SSOPs) and Good Manufacturing Practices (GMPs) and how to comply with them. For meat and poultry processors .SSOPs are the foundations of the plant's many food safety programs (Kevin, 2007).

2.4.2.7: Good Manufacturing Practices (GMPs):

Contain both requirements and guidelines for manufacturing of food and drug products in sanitary environment. The Food and Drug Administration has developed GMPs for all foods except meat, poultry, and egg products .Standard on Good Manufacturing Practices (GMP) for Poultry Abattoir applies to poultry transportation from farm to abattoir, humane slaughtering, good hygienic practices and thereafter distributing of poultry meat and products from abattoir to markets. GMP regulations are designed to control the risk of contaminating foods with filth, chemicals, microbes and other means during their manufacture (Kevin, 2007).

Good operating practice means documented procedures relating to practices that are required to achieve the fitness for intended purpose of the product and are appropriate to the business.

2.4.2.7.1 Sanitation Standard Operating Procedures (SSOPs):

SSOPs are the specific, written procedures necessary to ensure sanitary conditions in the food plant. They include written steps for cleaning and sanitizing to prevent product adulteration,. SSOPs are required in all meat and poultry processing plants. The GMPs can help guide the plant when the plants SSOPs are being developed. The SSOP procedures are specific to particular plants, but may be similar industry. All SSOP procedures must be appropriately documented and validated (Kevin, 2007).

2.4.2.7.2: GMPs categories

1. General maintenance of physical facilities

2. Cleaning and sanitizing of equipment and utensils

3. Storage and handling of clean equipment and utensils

4. Pest control

5. Proper use and storage of cleaning compounds, sanitizers, and pesticides

6. Employee training

7. Plant design

8. Quality assurance assessment these are the umbrella GMPs for all FDA-inspected food processing establishments regardless of size. Specific GMPs establish regulations for particular industries and products and are in addition to the umbrella GMPs.

For example, there are specific GMPs for seafood processors and dairy processors. Cross-contamination of food by foodhandlers is the most frequent cause of contamination. Employee hygiene is essential, because the hygienic condition and habits of workers determine the amount of cross- contamination from worker to food products. It cannot be overemphasized that clean, sanitary workers are necessary to produce clean, sanitary food products.

Examples of personal hygiene include washing hands, removing jewelry, and maintaining personal cleanliness. Also, the food processor should provide training for new employees in personal hygiene based on GMPs, and that training should be part of a formal, written training program that consists of instruction in proper handwashing, personal cleanliness, and sanitary hygiene.

2.4.2.7.3: Complying with GMPs regulations

As you may have noticed while browsing through the GMPs, some regulations are written so that compliance is easily evaluated. For instance, the regulation that "no pests shall be allowed in any area of the food plant" is clearly defined. If an inspector found a pest, such as a mouse, or evidence of a pest in the food plant, then there obviously is a violation of the regulation. However, some GMPs contain phrases such as "clean as frequently as necessary to protect against the contamination of the food." This vague regulatory. Language obviously is subjective. How often it is "necessary" to clean the processing line: daily, every two shifts, or when we think it needs it? Other GMPs might use the terms "adequately" or "sufficient," which are both subjective terms. These issues highlight the potential problems of determining how often to clean and sanitize. USDA-FSIS has developed more prescriptive requirements for meat and poultry processing. The SSOPs require processors to document that the sanitation program and personal hygiene practices are adequate to ensure that foods are produced under sanitary conditions. As a processor changes technologies or practices, changes in the SSOPs are necessary and must be documented with appropriate validation. Kevin Keener, Ph.D., P.E. (2009).

2.4.2.7.4: Operational SSOPs

These are established procedures that describe the daily, routine sanitary procedures that will be conducted during operations to prevent direct product contamination or adulteration. Established procedures for operational sanitation must result in a sanitary environment for preparing, storing, or handling any meat or poultry food product. Established procedures during operations might include, where applicable:

1) Equipment and utensil cleaning/sanitizing/disinfecting during production, as appropriate, at breaks, between shifts, and at mid-shift cleanup.

2) Procedures for employee hygiene, such as cleanliness of outer garments and gloves, hair restraints, handwashing, health, etc.

3) Product handling in raw and in cooked product areas. Meat and poultry plants are unique because they are required to develop, maintain, and adhere to written SSOPs. The plant must identify, by position, the officials who monitor daily sanitation activities, evaluate and document whether the SSOPs are effective, and take appropriate corrective action when needed. Finally, SSOPs must be routinely verified to ensure that they are working properly. Microbiological testing should be done periodically on food and noncontact surfaces to verify the effectiveness of the established procedures. SSOP records must be maintained on-site for 48 hours and maintained for a minimum of six months.

2.4.2.7.5: Meat and poultrySSOPs

The SSOPs for meat and poultry plants must meet the following regulatory requirements:

1. The plant has written SSOPs describing daily procedures that will be conducted before and during operations to prevent direct product contamination or adulteration. At a minimum, these procedures must address the cleaning of food contact surfaces, equipment, and utensils. The SSOPs state the frequency at which each procedure will be verified. 2. The SSOPs are signed and dated by plant management or plant owner. SSOPs should be reviewed periodically.

3. The plant must identify individual(s) who will be responsible for implementing and monitoring SSOPs and the daily sanitation activities.

4. Written records of SSOP activities along with corrective actions must be maintained for a minimum of six months (48 hours on site). Kevin Keener, Ph.D., P.E. (2009).

2.4.2.7.6: Plant Walk-through

A walk-through of the plant is an effective way to look at the variety of tasks involved in poultry processing. Usually chickens come into the processing plant by truck and in cages. In the receiving area, birds are stunned with an electric probe and live hung on a line that carries them through the kill room. They then pass to the scalders and pickers, where feet, head and feathers are removed. Birds then move to the eviscerating area where they are cut open. Liver and gizzards are removed and inspected prior to packaging, and lungs are removed. Viscera are inspected by U.S. Department of Agriculture (USDA) inspectors and then removed.

Next, birds move through final inspection and the wash. In accordance with USDA requirements, they are chilled to 40°F. When properly chilled, the birds move to the cutting and deboning lines where they can be placed on cones to move along a line where meat is removed according to the cut performed at each work station. Deboned meat is fine trimmed, and inspectors monitor both temperature and quality control. The meat is then packaged and shipped, either fresh or frozen, according to the purchaser's request. Whole fryers and roasters are not cut up.

In recent years there has been an increased demand for chicken over some other types of meat. Chicken is relatively lean and has fewer calories. Similarly, American consumers have demanded the convenience of "fast foods," precut and packaged meats and boneless chicken pieces. The poultry industry has had to institute changes to meet these public demands. Changes in the industry have heightened the need for attention to safety and health concerns. Particular safety and health concerns of this industry include the need for appropriate guards around the moving parts of machinery and the blades of saws, adequate ventilation, the use of personal protective equipment, and good housekeeping practices.

Better training for employees is essential to the betterment of employee safety and health. New employees and employees assigned to new jobs require additional training and observation. Training lines with close supervision allow employees to

gain job skills and become acclimated to their jobs while reducing the potential for injuries.

A number of conditions or practices in the poultry industry can be considered to pose hazards. The list includes tasks that could result in cuts or lacerations, repetitive motion disorders, slips and falls, exposure to cold and wet climates, exposure to dust, dermatitis, exposure to chemicals, and noise exposure. The remainder of this guide examines potential problems in the poultry industry and suggests preventive measures and possible solutions. (Cherie Berry Commissioner)

2.5: C0NDITI0NS 0F LICENSING F0R P0ULTRY SLAUGHTER-H0USES :

2.5.1: Structure

1. The buildings of the establishments, including their structures, rooms, and be of sufficient size to allow processing, handing, and storage of product in a manner that does not result in product adulteration.

2. The designed in a manner so as to allow one directionalflow to prevent cross-contamination between live poultry and dressed carcasses, the livepoultry handling area must be properly demarcated from the dressed carcasses area where finished products are handled.

3. The premises should be painted with durable and light colored paint that is easy to clean.

4. All ceilings must be as constructed and finished as to prevent condensation, leakage, and formation of moulds and can be easily cleaned.

5. Walls, floors, ceilings, partitions and doors must be constructed with smooth and durable materials

6. Windows and all openings must be constructed, maintained and meshed to prevent the entrance of dust and pests.

7. Floors must be made of non-slip materials.

8. There must be proper floor drainage and wastewater channeled into the sewerage system.

9. Adequate disinfecting facilities such as foot-bath must be provided at the entrance to the processing areas.

10. Adequate hand wash basins equipped with non-hand operated taps, liquid soap and disposable hand paper towels must be provided for food handler.

11. A separate slaughtering room for stunning, bleeding, scalding, waxing (for ducks only) and defeathering shall be provided. The bleeding and defeathering area shall be separated from the live bird's holding area by a wall.

12. An appropriate clean and hygienic system for removing feathers from the birds must be provided. Non-toxic wax can be used in defeathering .

13. A room for evisceration next to the slaughtering room shall be provided. Defeathered poultry from the slaughtering room shall enter the evisceration room by railing system or a chute.

14. Rooms or compartments in which edible product is processed, handled, or stored must be separate and distinct from rooms, handled, or stored. .

2.5.2: Reception Area of live Poultry (Unloading and Holding Bays)

1. All live poultry shall be delivered directly to the slaughterhouse. Live poultry are not allowed to be taken out of the slaughterhouse or sold without the prior permission or approvall of the AVA (2009).

2. Adequate sheltered holding facilities shall be provided for live birds awaiting slaughter.

3. The live poultry unloading area shall be so constructed that waste and dirty water are drained into manure sump, and no pollution shall occur to the neighboring unit.

4. The holding areas shall be well ventilated.

5. All poultry shall be given sufficient rest and water before slaughtering.

6. Live poultry shall be slaughtered within 24 hours of their arrival at the slaughterhouse.

2.5.3: Slaughtering of Live Poultry

1. Allpoultry shall be stunned to the right voltage and ampere depending on the size and weight of the birds before killing.

2. The method of slaughter shall be as humane as possible and approved by the AVA (2009).

3. All poultry shall be bled for about 90 seconds after killing.

4. Knife sterilizer(s) with hot water maintained at 82^0C shall be provided at the killing point, and the knife used sterilized regularly.

5. All poultry shall have the feathers completely removed before evisceration to prevent cross-contamination.

6. Evisceration of poultry from the side of the carcass is not permitted. Evisceration of poultry in water is strictly prohibited.

7. The cloaca of the poultry carcasses shall be properly ringed with an appropriate vent cutter. The ringed cloaca shall be completely removed together with the offal.

2.5.4: Inspection Station/Point

1. An inspection station/point must be provided on-line after the evisceration process.

2. Adequate inspection mirror(s) and knife sterilizer(s) with hot water maintained at 82^0C shall be provided at the inspection station/point.

3. Adequate veterinary inspector(s) must be engaged to carry out or supervise ante-mortem and post-mortem inspection and examination of the carcasses.

2.5.5: Chiller Tank

1. Dressed poultry shall be chilled to 4^0C or below within 11/2 hours of evisceration.

2. The flow of the water in the chiller tank shall be on the opposite direction where the dressed poultry is moving.

3. The contact time of the dressed poultry in the spin chiller shall be at Least 20 minutes.

4. Ice used for processing and chilling of dressed poultry must be manufactured from potable water.

5. Utilized ice must be stored and protected from contamination.

2.5.6: Tagging of dressed & thawed poultry

1. All freshlyslaughteredpoultry carcasses shall be individuality tagged. 2. The tags shall carry the name of the slaughter-house and the date of slaughter. Post-dated tagging is not permitted.

3. Service slaughter-house slaughtering on behalf of clients shall indicate both the client's name and the slaughterhouse name on the tags.

4. Slaughter-houses with prior permission from AVA (2009) to carry out thawing of frozen poultry and poultry parts shall ensure that the thawed poultry/poultry parts are tagged and labeled.

2.5.7: Chiller & Freezers

1. Adequate built-in chiller(s) and freezer(s) must be provided for storage of poultry and its products.

2. The temperature of chiller must be maintained between 0 ^{0}C – 4 ^{0}C and that of freezer must be –18 ^{0}C or below. AVA (2009).

3. Chillers & freezers must not be over Loaded beyond their designated capacity.

4. Chillers and freezers must be maintained in a sanitary condition at all times and there must be no accumulation of ice formation in the chambers. AVA (2009).

2.5.8: Thawing Room

1. Thawing or processing of frozen poultry or its parts is not permitted unless with the prior approval from AVA (2009).

2. The temperature of the thawing and processing rooms must be maintained at around 12 ^{0}C.

2.5.9: Cutting Room

1. Any further cutting up of the dressed poultry shallonly is carried out in a separate room approved by the Authority, around 12 ^{0}C – 15 ^{0}C.

2. A knife sterilizer with potable water maintained at 82^{0}C must be provided.

2.5.10: Packing Room

1. This room must ideally be Located between the cutting room and the finished product cold room.

2. The room temperature 12 ^{0}C – 15 ^{0}C.

2.5.11: Storage Rooms for non-food items

1. A store room for wrapping/packaging materials must be provided.

2. Chemicals, detergent and any hazardous materials separate from store

2.5.12: Equipment Washing Room

1. A room of washing of utensils and equipment. .

2. Hot water must be provided in this room for the cleaning.

2.5.13: Equipment and Utensils

1. Equipment and utensils must be designed and constructed to be durabIe

2. 0nIy food grade Lubricants must be used: a) Safe and non-toxic; b) Durable, corrosion-resistant, and non-absorbent; c) Sufficient in weight and thickness to withstand repeated washing; d) Finished to have a smooth, light-colored, easilycleanable surface.

2.5.14: Lighting

Lighting of good quality and sufficient intensity must be provided in areas where food is process

2.5.15: Ventilation

1. Ventilation must be adequate to control odors, vapors.

2. Ventilation hood systems and devices must be sufficient in number and capacity.

3. Heating, ventilating, and air conditioning systems must be make-up air intake and exhaust vents do not cause contamination of food.

2.5.16: Changing Rooms and Toilets

1. Its sufficient in number, ample in size, in a sanitary condition and in good repair at all times to ensure cleanliness.

2. Toilets must not open directly into any place where food products are prepared.

3. Changing rooms must be separate from toilets.

4. Hand wash basins equipped with non-hand operated taps, Liquid detergent and disposable hand paper towels must be provided adjacent to the toilets.

2.5.17: Welfare Room:

1. Shall be provided for the workers to take their meals, rest and for recreational purposes.

2. The room must be separated and away from the rooms and areas in which products are processed.

2.5.18; Disposal of Waste

1. Sewage system separates from aII other drainage lines to prevent backup of sewage into areas where product is processed.

2. Trimmings and waste must be disposed of regularly during and after processing.

3. Effluent or sewage lines must not pass directly over unless they are adequately controlled.

2.5.19: Hygiene of Premises

1. Its including the walls, floors, working tables, utensils and equipment must be kept in good repair and in a clean and hygienic condition at all times.

2. Good house-keeping, Good Manufacturing Practices (GMPs) and Good Hygiene Practices (GHPs).

3. Workrooms must be washed and cleaneddaily and disinfected.

4. All equipment, tables, utensils and protective clothing must be cleaned before and after operations and cleaned and disinfected whenever they become contaminated and at the end of the working day.

2.5.20: Employer's responsibilities

1. Provide training in food handling and personal hygiene.

2. Conduct regular inspections of employees' hygiene and hygienic work habits. Violations should be handled as disciplinary violations.

3. Sanitary facilities and supplies, includes ample quantities of soap, disinfectant, working sinks, hairnets, etc.

Personnel Hygiene

1. Cleanliness: All persons working in contact with product, food-contact surfaces.

2. Clothing such as aprons, frocks, and other outer clothing worn by persons who handle product.Clean garments, head covers and boots must be worn at the start of each working day and garments to prevent contamination.

3. Disease control: Appears an infectious disease, open lesion, including boils, sores, or infected wounds, any other abnormal source of microbial contamination must be excluded from any operations process.

4. Personnel must clean their hands using for food preparation used for the disposal of mop water and similar liquid waste.

5. Personnel must keep their fingernails trimmed, filed.

6. While preparing food, personnel must not wear jewelerson their arms and hands. Smoking, chewing of gum or tobacco, littering and other undesirable behavior in the premises is prohibited.

7. Hands must be washed before commencing work, after using toilets, when contaminated.

2.5.21: Cold Chain System

All dressed poultry shall be delivered in refrigerated vehicles that are so equipped as to be capable of maintaining the dressed poultry at a temperature of 4^0C or below (for chilled poultry), -18^0C or below (for frozen poultry) and with a core temperature not exceeding 7^0C (for chilled poultry), -12^0C (for frozen poultry) during transportation.

2.5.22: Medical Examination of Food Handlers

1. AII workers handling exposed food and/or who clean food equipment, utensils, etc, are required to be examined and found medically healthy by a medical practitioner registered under the Medical Registration Act before he/she starts working in the company

2. No person, known or suspected to be suffering from, or to be a carrier of, a disease.

2.5.23: Pest Control

1. Slaughter-houses must pest management program to prevent the harborage & breeding of pests on the grounds.

2. Pest control substances used must be safe and effective under the conditions of use.

3. Exclude vermin, e.g. rats, mice, cockroaches and flies from entry and harboring in the premises and any delivery vehicle.

4. The service of a professional pest control company must be required if the pest control carried out by the slaughter-house is found not effective.

5. No pets including birds and animals must be kept within or around the premises.

2.5.24: HACCP (Hazard Analysis Critical Control Point) or Similar Food Safety Systems

1. All slaughter-houses should implement HACCP or similar food safety management system to control all food hazards in the slaughtering/manufacturing processes to ensure the production of safe and wholesome food for human consumption.

2. There must be proper documentation of the establishment's sanitary Standard 0perating Procedures (SS0Ps). A proper recall system or program must be in place to remove or correct marketed consumer products that violate the law.

2.5.25: Personnel Training

1. The necessary knowledge and skill to enable them to handle poultry and its products hygienically. They could either attend courses/seminars/workshops

conducted by reputable institutions/companies or any in-house training sessions conducted by trained personnel.

2. All poultry slaughter-houses should ensure that individuals have received adequate and appropriate training in the design and proper application of a HACCP system and process control.

2.5.26: Restricted use of Premises:

1. 0nly the type of poultry as stated in the license is allowed to be slaughtered in the slaughterhouse.

2. The number of poultry to be slaughtered in each premise is subject to the approval of the AVA (2009), which would be based on the capacity, and Iine speed of the slaughtering plant.

3. No further washing, drying, processing or storing of feathers is permitted inside the slaughterhouse.

4. No portion of the slaughter-house shall be used as living quarters or for other activities other than those approved by the AVA. (2009).

5. The licensee shall ensure that only authorized personnel are allowed into any area where the poultry is slaughtered, processed Submission of Documents

6. The licensee of a poultry slaughter-house shall furnish a daily report stating the number and type of poultry for slaughter, provide all Customs Clearance Permits (CCPs) and all relevant import documents to any authorized officer* who conducts inspections at the slaughter-house. (AVA 2009).

2.6: Control of Bacteria in Poultry Meat:

2.6.1: Prevention of contamination:

It is important to avoid the contamination of meat poultry whenever possible. This includes inadvertent contamination from live animal, processing producers and equipment, employees, and the environment. (NACMCF, 1999) Contamination can be minimized or avoided altogether by following appropriate sanitation procedures, good manufacturing practices, and produces for employee hygiene (NACMCF, 1999) the term "cross-contamination" generally refers to the transfer of organism from a contaminated source to a previously uncontaminated surface. A particular concern is cross-contamination of ready- to eat foods with not-ready- to eat (raw or partially cooked) meat or poultry, or with drippings from not –ready-to –eat meat or poultry. It is particularly to important to ensure complete separation of not-ready-to –eat and ready –to-eat products. Instead, there is a growing emphasis on the application of preventative measures within the industry and there is now much reliance on the HACCP system for controlling food borne pathogens in poultry processing (NACMCF, 1999).

2.6.2: Restrictions of Growth:

To keep the overall number of bacteria very low in order that concern about pathogens can be minimized. Temperature, acidity, salt and drying, and combinations of these can be used to restrict growth of pathogens (NACMCF, 1999).

2.6.2.1: Temperature

The growth of most bacteria can be slowed i.e. controlled, by maintaining the product at refrigeration temperatures (less than 41F^{o}), or by freezing. Some bacteria survive freezing, so freezing cannot be considered a method to

eliminate them. On the other hand, holding products at higher temperature (greater than 130F$^{o)}$ also restricts the growth of bacteria (NACMCF, 1999).

2.6.2.2: Acidity

Fermentation restricts the growth of bacteria of public health concern by increasing the acidity i.e. lowering the PH, of the product. Generally, a PH of less than 5 will severely restrict or completely stop the growth of harmful bacteria can survive in acidic conditions, so fermentation alonecannot be relied upon to completely eliminate all harmful bacteria or reduce their number to a tolerable level (NACMCF, 1999).

2.6.2.3: Salt and Drying

Some products contain high levels of salt. Salt and low moisture content in a product can be effective in controlling growth of some organisms (e.g. *Staphylococcus aureus*) survive in high salt environments (NACMCF, 1999).

2.6.3: Destruction of Bacteria

Most pathogenic bacteria, including: *Salmonella species E.coli 0157:H7, Listeria monocytogenes, and Campylobacter species*, can easily be destroyed using a rather mild cooking process maintaining a minimum temperature within the range of 130F^{o} to 165F^{o} for specific amount of time. However, cooking at the temperature range and for specified dwell time will not destroy the heat resistant forms (spores) of certain bacteria,

nor will some types of toxins be destroyed if they have already been formed in the product, Thermal processing (canning) at a minimum retort temperature of greater than 249F^{o} for a specific amount of time is necessary to destroy most spores and toxins (NACMCF, 1999).

2.6.4: Sanitation

Some bacteria, such as listeria; include*L. monocytogenes*; can be found in the processing environment. Although most forms of Listeria are not pathogenic, *L/ monocytogenes* are a pathogen organism. This emphasizes the need for adequate sanitation, not only of the equipment, but also the floors (NACMCF, 1999). Employee's hygiene, air flow, and traffic flow of people and equipment between areas used for not-to-eat processing is very important and should be strictly controlled (NACMCF, 1999).

2.6.5: Personnel Hygiene

1. **Cleanliness**: All persons working in contact with product, food-contact surfaces, and product-packaging materials must adhere to hygienic practices whiIe on duty to prevent aduIteration of product.

2. **Clothing** such as aprons, frocks, and other outer clothing worn by persons who handle product must be of material that is disposable. Clean garments, head covers and boots must be worn at the start of each working day and garments must be changed during the day as often as necessary to prevent contamination.

3. **Disease control:** Any person who has or appears to have an infectious disease, open lesion, including boiIs, sores, or infected wounds, or any other abnormal source of microbial contamination must be excIuded from any operations which couId resuIt in product adulteration untiI the condition is corrected.

4. **Personnel** must clean their hands using non-hand operated washing facilities and may not clean their hands in a sink used for food preparation, used for the disposal of mop water and similar liquid waste.

5. **Personnel** must keep their fingernails trimmed, filed, and maintained so the edges and surfaces are cleanable and not rough.

6. **While preparing food**, personnel must not wear jewellery on their arms and hands. 0ther loose and uncovered jewellery or personal effects that pose any contamination risk must not be worn in food handling areas.

7. **Smoking**, chewing of gum or tobacco, Iittering and other undesirabIe behaviour in the premises are prohibited.

8. **Hands** must be washed before commencing work, after using toilets, when contaminated, and as frequently as necessary

Chapter Two

Materials and Methods

3. Samples of the study

3.1: Sources of samples

The swabs were collected from two types of poultry slaughterhouse, Automatic abattoir of poultry and from traditional slaughtering process in the period of frist six months in 2017

3.2: Collection of samples:

A total of 180 swab samples were collected from carcasses of broiler chickens and employees in two types of slaughtering processes, Automatic slaughter house and Traditional slaughtering process. Samples were collected directly from broiler chickens lived in closed system houses in two types of slaughtering processes; the collection of samples was done during six months - every thirty samples collected monthly. At frist sixth months in 2017

3.2.1: Collection from an Automatic Slaughter house:

Ninety (90) samples were collected from an Automatic Slaughter house in Khartoum State (HACCP SYSTEM):

A total of 30 sawb samples were taken from six critical points, 5 samples from each critical point: Scalding (neck), Defeathering (skin), Evisceration (abdominal reigon), After Washing (skin), After Chilling (skin),) and the sixth one from hands of employees as shown in Appendix1.From the same six ctritical points Three replicates were done.

3.2.2: Collection from Traditional Slaughter Processing:

Ninety (90) swab samples were collected from traditional slaughtering processes in Khartoum State (HACCP SYSTEM):

A total 30 sawb samples were taken from six critical points, 5 samples from each critical point: Scalding (neck), Defeathering (skin), Evisceration (abdominal reigon), After Washing (skin), After Chilling (skin) and the sixth one from hands of employees. as shown in Appendix 2. From the same six ctritical points Three replicates were done.

3.3: Submission of samples:

The collected swabs were transported promptly on ice pot to Laboratory of Microbiology (College of Veterinary Medicine, Sudan University of Science and Technology).

3.1.1: Collection method

The swab samples collected from broiler meat on surface in space $2cm^2$ were obtained by sampling technique from carcasses of broiler chickens. The five samples were taken from each six critical control points CCPs. (Scalding, from upper part of neck, at Defeathering from upper part of thorathix, at Evisceration from lower part of the interinal obdominal cavity , After Washing from upper part of skin of hind limb , After Chilling from upper part of skin of hind limb , and Hands of Employees). Samples were collected in sterile tubes and preserved as dry samples in ice and transferred to Laboratory of Microbiology.

3.1.2: Sample preparation:

Samples for microbial assessment and identification of contaminants were taken to microbiology lab for microbial assessment and identificants.Glassware such as petri-

dishes and test tubes (metal or cotton stopped) were sterilized in a hot air oven, at 160^0C–170^0C for one hour. Tubes and flasks capped with polypropylene covers and screw-capped bottles were sterilized by autoclaving according to Barrow and Feltham, (2003).

3.2: Asepsis and sterilization

3.2.1: Flaming

Flaming was used to sterilize slides, cover slips and glass rods.

3.2.2: Red Heat

It was used to sterilize l wire loops, needles and spatulas by holding them over Bunsen burner flame until became red.

3.2.3: Hot air oven

It was used for glassware such as pipettes, Petri dishes, tubes and flasks, which were sterilized at 160^0C for 1h.

3.2.4: Moist Heat (autoclaving)

It was used for sterilization of media, solutions, screw-capped bottles, rubber-Stoppered flasks, and plastic ware which were sterilized at 121^0C for 15 min (15 Ib/inch2) and 110^0C (10 Ib/inch2) for 10 min for sugar media.

3.2.5: Boiling

It was used for dissolving the agar-containing media.

3.2.6: Irradiation

Ultraviolet irradiation for 20 min was used to sterilize media pouring room.

3.2.7: Disinfection

Alcohol (70%) was used to disinfect working benches in the laboratory and media preparation room.

3.3: Reagents and indicators

3.3.1: Reagents

All reagents were obtained from British Drug House chemicals (BDH), London and prepared according to Barrow and Feltham (1993).

3.3.1.1: Normal saline

Normal physiological or isotonic saline was prepared as described by Oxiod Manual by dissolving 8.5 grams of sodium chloride in one liter of distilled water to obtain 0.85% concentration.

3.3.1.2: Hydrogen peroxide (H_2O_2) It was prepared as 3% aqueous solution, protected from light and stored in a cold place. It was used for catalase test.

3.3.1.3: Oxidase test reagent

A fresh aqueous solution of 1% tetra-methyl–p–phenylene diamine dihyrochloride was added to a fresh solution of 1% ascorbic acid. This was used to impregnate filter papers, which were dried at 50°C and used for oxidase test.

3.3.1.4: Potassium hydroxide

This was obtained from Hopkins and Williams, London. It was prepared as 40% solution and used for Voges-Proskaeur (VP) test.

3.3.1.5: Kovac's reagent

This reagent was composed of 5g para-dimethyl-amino benzaldyhyde, 75 ml amyl alcohol and 25 ml concentrated hydrochloric acid, which was added carefully. The reagent was stored at 4^0C for later use in indole test.

3.3.1.6: Lead acetate paper

Filter paper strips, 4-5 mm wide and 50-60 mm long were impregnated with lead acetate saturated solution and then dried and used for hydrogen sulphide test.

3.3.1.7: Sodium hydroxide and hydrochloric acid

Sodium hydroxide (1N) and hydrochloric acid (1N) were used to adjust the pH of different media.

3.3.1.8: Methyl red solution

This solution was prepared by dissolving 0.04g of methyl red in 40 ml ethanol and the volume was made up to 100 ml with distilled water.

3.3.1.9: Alpha-naphthol solution

This solution was obtained from Hopkin and Williams, London. It was prepared as 5% solution and used for VP test.

3.3.1.10: Gram's stain reagents

3.3.1.10.1: Lugol's iodide

Potasium iodide 20 g

Iodine 10 g

Distilled water 1 liter

Potassium iodide was weighed and dissolved in about a quarter of water. Iodine was added to the potassium iodide solution and mixed well; the solution was made up to 1 liter with distilled water, mixed well and then stored in dark place at room temperature.

3.3.1.10.2: Crystal violet

Crystal violet 20.0 g

Ammonium oxalate 9.0 g

Ethanol up to 95 ml

Alcohol was added to the crystal violet and mixed well until the dye was completely dissolved. Ammonium oxalate was weighed and dissolved in about 200 ml of distilled water and then it was added to the stain and made up to one liter with distilled water and mixed well; then stored at room temperature.

3.3.1.10.3: Acetone-alcohol-decolorizer

Acetone 500 ml Ethanol or methanol, absolute 475 ml Distilled water.

25 ml Distilled water was mixed well with alcohol, acetone was measured and added immediately to alcohol solution, mixed well and stored at room temperature.

3.3. 1.10.4: Dilute carbol fuchsin

One volume of strong carbol fuchsin was added to 10-20 volumes of distilled water. Strong carbol fuchsin consists of two solutions;

Solution A: ten grams of basic fuchsin mixed with 10 ml of ethanol (95%) and dissolved in stoppered bottle and kept at 37^0C over night. Solution B: five grams of phenol were mixed with 100 ml of distilled water and shaken to dissolve. Strong carbol fuchsin was prepared by pouring 10 ml of solution

An in 100 ml of solution B.

3.3.2: Indicators

3.3.2.1: Anderade's indicator

It is composed of acid fuchsin (5g), distilled water (1liter) and 1N-NaOH (150 ml). It was prepared by dissolving the acid fuchsin in the distilled water and then the 150 ml of alkali solution were added, mixed and allowed to stand at room temperature for 24 hour with frequent shaking until the color changed from red to brown.

3.3.2.2: Bromothymol blue

This indictor was prepared by dissolving 0.2 gram of bromothymol blue powder in 100 ml distilled water.

3.4: Bacterial Culture Media:

All media were prepared according to the Oxoid Manual for culture media Ingredients and Barrow and feltham (2003) as fallows:

3.4.1: Solid Media:

3.4.1.1: Blood Agar (OxiodCM0055) Media: is used with blood for isolation &cultivation of wide variety of fastidious microorganisms

3.4.1.2: MacConkey's Agar (OxiodCM0007) Media(: is commonly used media to culture & indentify gram negative bacilli (especially bacteriaceae.) this medium 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 4C° before used .

3.4.1.3: Nutrient Agar(OxiodCM0003) Media: is a basic culture medium used to subculture organisms for maintenance purposes or check the purity of subcultures from isolation plate'sprior biochemical or serological tests to detect Total Bacterial Count.

3.4.2: Semi Solid Media:

Hugh and leifson,s (O.F) medium

The medium was prepared by dissolving 10.3 grams of medium in 1 liter of distilled water by heating, and the pH was adjusted to 7.1.filltered bromothymol blue (0.2% aqueous solutions) was added and then sterilized at 115o C for 20 minutes. Sterile solution of glucose was added aseptically to give final concentration 1%, mixed and distributed aseptically into sterile tubes.

3.4.3: Liquid media*:*

3.4.3.1: Nutrient Broth(oxoid CM0001):

This medium was prepared by dissolving 13g of the medium in 1 liter of distilled water. The pH was adjusted to 7.4, distributed into screw-capped bottles 5 ml each and sterilized at 121o C for 25 minutes.

The basal medium for preparation of the media it contains:

Meat extract infusion or digest =10 gram

Sodium chloride =5gram

Distal water =1000ml

Is general usage medium for large variety of microorganisms without particular nutritional requirements?

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

3.4.3.2: Peptone water:

May be 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.

Thirteen grams of dehydrated nutrient broth was added to 4 grams of agar and dissolved in 1 liter of distilled water by boiling; the pH was adjusted to 7.4, distributed in 5 ml amounts in tests tubes containing Craig-tubes and sterilized by autoclaving at 121o C for 15 minutes.

Peptone water sugars

Nine hundred ml of peptone water was prepared and pH was adjusted to 7.1-7. 3 before 10 ml of Andrade s indicator was added. Ten grams of the appropriate sugar was added to the mixture, distributed into tubes 5 ml in each one. The peptone water was sterilized by autoclaving at 110oC for 10 minutes. The sugars used were Glucose, mannose, mannitol, maltose, sucrose, lactose, fructose, raffinose, and trehalose.

.3.2.2 Motility medium:

Ingredients	gm/liter
Peptone	10.0
Sodium Chloride	5.0

3.4.4: Selective Media:

3.4.4.1: Mannitol Salt Agar (OxoidM118-500G) Media:

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

Ingredients	gm/liter
Lab-Lemco Powder	1.0
Peptone	10.0
Mannitol	10.0
Sodium Chloride	75.o
Phenol red	0.025
Agar	15.0

3.4.4.2: Milk Palate Agar

Ingredients	gm/liter
Tryptone	5gm
Yeast Extract	2.5gm

Glucose	1gm
Antibiotic Free Skim Milk	1gm
Agar	10gm

3.4.4.3: DexyoCholateCitrate Agar (DCA) Agar Oxoid M06500G:

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.

Ingredients	Ingredients
Special peptone	10.0
Sodium Citrate	10.0
Sodium Thiosulphate	5.0
Lactose	5.0
Sodium deoxycholate	5.0
Sucrose	2.5
Neutral red	0.03
Special peptone	12.0
Agar	10.0

3.4.4.4: EMB Agar (Eosin Methylene Blue Agar)OxoidM0500G

For the isolation, cultivation, and differentiation of Gram-negative enteric bacteria based on lactose fermentation. Bacteria that ferment lactose, especially the coliform bacterium Escherichia coli, appear as colonies with a green metallic sheen or blue-

black to brown color. Bacteria that do not ferment lactose appear as colorless or transparent, light purple colonies.

Ingredients	**gm/liter**
Dipotassium MonohydroenPhosphate	2.0
Peptone	10.0
Lactose	10.0
Methylene Blue	0.065
Eosine Y	0.4
Agar	15.0

3.5: Bacterial Count:

3.5.1: Plate Count Agar:

A medium for the enumeration of cultured microorganisms used a colony count technique.

3.5.1.1: Bacterial Cell Counting Techniques:

It is necessary to enumerate bacterial cells in diagnostic and food hygiene procedures. Viable bacteria were capable of multiplication with production of visible colonies on or in agar media, in viable counting method the assumptions made that one well-spaced, bacterial cell give rise to one colony .Bacterial colonies rather than bacterial cells are counted in most of the methods (A non1982).

3.5.1.2: Viable Plate counts:

The bacterial count was done according to Miles and Misra(1938).

A standard plate count method was used to determine the number of viable bacterial cells per unit volume of sample, to determinate the number of viable bacterial cells per milliliter of aliquid sample a fixed 0.2 ml volume of sample was transferred to a plate, the solution spreaded across the plates and the colonies were counted after incubation. The colonies, referred to as colony forming units (CFUs), on the plate were determined. If the sample contained over one thousand cells per unit volume then it would produce too many CFUs to a counted accurate on the plate. These samples were diluted in sterile media before 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 diluted the sample serially. The plate with countable number of colonies was selected to count when used standard size Petri - dishes ,a countable plate was one with between 30 and 300 CFUs .Dilutions with fewer than 30 colonies were easy counted, but produced in accurate results since one or two contaminated colonies could caused significant over estimated of the cell count. After the colonies were counted the concentration of the cells in the plates dilution was determined by dividing by the amount plated . Once the concentration of the cells at the specific dilution determined, the concentration in the original sample was calculated after divided by the total dilution (used Aseptic technique).

3.5.1.2: Procedures of microbial analysis:

Add 5ml peptone water in sterile test tubes and put its in Autocalve at 100^0C for 1hour, till rich room temperture then put the collected samples in this tubes(orgional sample)

Serial dilutions were prepared from normal saline solution included as follows:

Serial folds dilutions in sterile test tubes every one contained 9 ml normal saline, then 1ml by micro pipette from orgional sample included bacteria, added in frist tube included 9ml of normal saline dilutee. From first tube dilution1/10 had taken 1ml by micro pipette added to second tube included 9ml of normal saline dilutee.Dilution1/100 concentration, till the dilutions reached 1/100000 concentration.

From the 4^{th} tube (1/10000) and5 th 1ml (1/100000) was taken by micro pipette fom each, then added to the surface of two petri-dish containing nutrient Agar Media count plate and incubated (overnight) at $37C^{o}$ for 24 hours.

3.5.1.3: Colonies Count:

In test samples colonies were counted from each petri dishes (180 samples) which growth in Nutrient Agar Media in all stages of automatic and traditional slaughtering process, according to Miles and Misra (1938), the average was multiplied by 50 to obtain a figure for the bacteria/ml in the original sample and by the reciprocal of dilution factor. (4^{th} tube (1/10000) and (5th tube (1/100000), as shown in Appendix1and2

3.5.2: Bacterial Isolation and Indenitifaction:

The morphology of colonies on agar media were examined microscopically, smears were made on to clean slides fixed with heat and subjected to Gram stain and examined under oil lens immersion to ensue purity of the culture

3.5.2.1: Bacterial Isolation: The colony characteristics of all isolates (shape, size, consistency, opacity, pigments and type of growth on different media) were observed and recorded. Cultures on solid media as shown in appendix (3) were examined with naked eye for growth and colonies morphology as well as any change

in the media. The liquid media were examined with naked eye for turbidity and color change in case of *Salmonella* isolation as shown in appendix (3)

In test samples we were taken swabs from each orginal test tubes (180 samples) in peptone water and cultured in solid media, after incubation 24 hours, were examined with necked eye for growth morphology as well as change in the media. In two types of an automatic and traditional slaughtering process, according solid media and types of Bactria (*Salmonella spp., Escherichia coli and Staphylococcus aureus)* as follows in appendix 3

3.5.2.2: Identification:

The purified isolates were identified according to criteria described by Barrow and Fetham (2003). This included staining reaction, organism morphology, growth condition, colony characteristics on different media, and biochemical characteristics.

3.5.2.2.1: Microscopic Examination

A smear was made from each colony type of primary cultures and from purified colonies, fixed by heating and stained by Gram`s method. Then the stained smears were examined microscopically by oil immersion lens. The smears were examined for cell morphology and arrangement, presence of capsule and staining reaction.

3.5.2.2.2: Gram`s staining method:

1. Crystal violet was added to the fixed smear for 30 seconds.

2. Washed with distilled water.

3. Lugol`s iodine was added for 30 seconds.

4. Decolorized with acetone-alcohol for 2-3 seconds.

5. Washed with distilled water.

6. Counter stained with dilute carbol fuchsin for 30 seconds.

7. Washed with distilled water.

8. Dried with filter paper and examined under microscope by oil immersion objective lens. Gram-positive bacteria appeared purple, while Gram-negative bacteria appeared red. (Cruickshank et al, 1973)

3.5.2.2.3: Biochemical testing

The following biochemical tests were conducted and performed according to Barrow and Feltham (2003).

3.5.2.2.3.1: Primary tests

3.5.2.2.3.1.1: Catalase test

A drop of 3% hydrogen peroxide was placed on a clean slide and a colony of test organism, cultured on nutrient agar, was picked by glass rod and added to the drop of H_2O_2. A positive result was indicated by immediate production of air bubbles.

3.5.2.3.2.1.2: Oxidase test

A strip of filter paper, which was soaked in 1% solution of tetra-methyl–p–phenylene diamine dihydrochorde (oxidase reagent) and dried in hot air oven, was placed on a clean glass slide by a forceps. A fresh young test culture from Nutrient Agar was picked off with a sterile glass rod and rubbed on the filter paper strip. If blue–purple color developed within 5-10 seconds, the reaction was considered positive.

3.5.2.2.3.1.3: Oxidation Fermentation (O/F) test

The test organism was inoculated by stabbing with a straight wire into two test tubes of Hugh and Leifson's medium. To one test tube, a layer of melted sterile soft paraffin oil was added to seal the medium from air; both test tubes were incubated at 37°C and were examined daily for up to two weeks. Yellow color in the open tube only indicated oxidation reaction, yellow color in both tubes indicated fermentation reaction, while green in both tubes indicated that no oxidation or fermentation of glucose. **3.5.2.2.3.1.4: Motility test**

By a sterile straight wire, a small piece of colony was picked and stabbed in the center of the semi-solid agar in the Craigie tube. This preparation was incubated at 37^0C overnight. The growth outside Craigie tube and turbidity in the medium indicated that the organism is motile.

3.5.2.2.3.1.5: Fermention tests

Tubes of glucose sugar medium were inoculated with the test culture and incubated for up to 7 days at 37^0C. Production of acid and gas was indicated by the presence of empty space in the inverted Durham's tube

3.5.2.2.3.2: Secondary tests

3.5.2.2.3.2.1: Voges-Proskauer (VP) test

Pure test culture was inoculated in a test tube or screw–capped bottle containing glucose phosphate broth and incubated at 37^0C over night. 0.2 ml of 40% KOH and 1.6 ml of 5% alpha-naphthol solution were added to one ml of culture, shacked and the tubes were placed in a slope position and examined. Positive test was indicated by a strong red color within half an hour.

3.5.2.2.3.2.2: Sugars fermentation test

Carbohydrate medium was inoculated with 24 h peptone water culture, then incubated at 37^0C and examined daily for up to 7 days. Production of acid was indicated by appearance of a reddish or pink color.

3.5.2.2.3.2.3: Methyl red (MR) test

Tubes containing glucose-phosphate peptone water medium were inoculated with 24 h-peptone water cultures and then incubated at 37^0C for 24 h. Two drops of methyl red reagent were added, shaken well and examined. Appearance of bright red color indicated a positive reaction whereas orange yellow color indicated a negative reaction.

3.5.2.2.3.2.4: Hydrogen sulphide (H_2S) production test

H2S production was also examined by lead acetate paper method. A tube of peptone water medium was inoculated by the test organism and a lead acetate paperwas inserted between the cotton plug and the tube, incubated at 37^0C and examined daily for a week. Blackening of the paper was considered as a positive result.

3.5.2.2.3.2.5: Citrate test

The test organism (grown on Nutrient Agar) was heavily inoculated into a slope of Simmons`s citrate agar, then incubated at 37^0C and examined after 24 h and daily for up to 7 days. Blue color and growth of the organism indicated a positive result; while, green color and no growth indicated a negative result.

3.5.2.2.3.2.6: Indol test

The test organism was cultured into peptone water which contains tryptophan and incubated at 37^0C for 48 h. One milliliter of Kovac`s reagent, which contains 4- P-dimethylamine benzaldehyde was run down along side of the test tube. Appearance of a pink color in the reagent layer within a minute indicated a positive reaction.

3.5.2.2.3.2.7: Coagulase tube test

This technique is used to detect the free coagulase proceduction by the propagated bacteria. Fresh rabbit plasma was diluted 1:10 in physiological saline. An amount of 0.5 ml of diluted plasma was placed in a sterile test tube and 0.5 ml of overnight growth culture of the test organism was added. The tube then was incubated at 37^0C and examined after 1, 3 h, and 24 hours. A positive test was indicated by coagulation of the tube content.

3.5.2.2.3.2.8: Malonate test

Malonate broth medium was lightly inoculated and incubated with the test organism at 37^0C for 24 h. A positive reaction was indicated by a deep blue color

3.5.2.2.3.2.9: Urease Test:

This test is preformed 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 35-37^0C 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

Bacterial Indentification by Biochemical test *(Arora etal2014)* as follows in appendix 4.

3.5.5: Statistical Analysis

Data analysis: The data were analyzed with SPSS software (Statistical package for social science version 20, IBM/SPSS). Descriptive statistics were used to analyze the data. In addition, all TVCs of bacteria were converted to log10 CFU/ml for analysis. ANOVA was performed. Statistical significance was set at P- value of ≤ 0.01

Statistical analysis: The averages of plate counts were converted to log CFU/chicken. One way analysis of variance (ANOVA) was performed to compare differences of bacterial population or relative abundance among groups with JMP® Genomics 7.0 (SAS Institute Inc., Cary, NC) at $P < 0.01$

Chapter Three

Results

Total Bacterial Count (TBC) in automatic slaughter house and traditional slaughtering processes at six different operational processes (scalding,

defeathering, evisceration, after washing, after chilling, and hands of employees).

The study also revealed the isolation and indentification of three types of bacteria, namely: (*Salmonella spp., Escherichia coli and Staphylococcus aureus)* at same different operational processes (scalding, defeathering, evisceration, after washing, after chilling, and employees) As shown in table 1 and Fig 1: Total Viable Count (TVC) revealed that the highest contamination was detected in the traditional poultry slaughtering processes and lowest contamination was detected in an automatic poultry house process. There was significant difference at p-value ($P \geq 0.01$) in all operation processes.

At scalding point (TVC) showed high contamination (mean (log_{10}CFU/ml**)** 7.28 ±0.11) in traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 7.01 ±0.21) in an automatic poultry slaughter house.

Defeathering point showed high contamination (mean (log_{10}CFU/ml**)** 7.43 ±0.08) in traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 7.09 ±0.13) in an automatic poultry slaughter house.

Evisceration point showed high contamination (mean (log_{10}CFU/ml**)** 7.43 ±0.16) in traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 6.86 ±0.15) in an automatic poultry slaughter house.

After washing point showed high contamination (mean (log_{10}CFU/ml**)** 7.36 ±0.11) in Traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 6.68 ±0.15) in an automatic poultry slaughter house.

After chilling point showed high contamination (mean (log_{10}CFU/ml**)** 7.30 ±0.14) in traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 6.84 S ±0.29) in an automatic poultry slaughter house.

Employee's hands showed high contamination (mean (log_{10}CFU/ml**)** 7.37 ±0.16) in traditional poultry slaughtering process and low contamination (mean (log_{10}CFU/ml**)** 6.74 ±0.18) in an automatic poultry slaughter house.

The study revealed a statistically significant difference at p-value (P≥0.01) in different operational points in the two types of slaughter houses automatic and traditional slaughtering process in Khartoum State.

Table 1: Means of Total Viable Counts of Bacteria (log_{10} CFU/ml) and standard diviation at Different Operational Points:

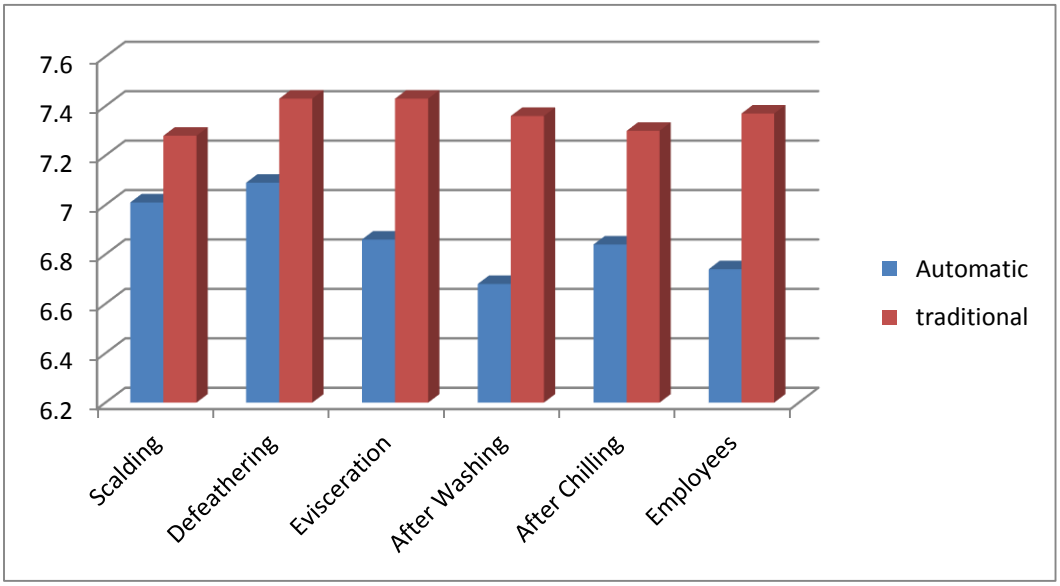
Control	Type of slaughter house		
Critical points	Automatic	Traditional	Significant
Scalding	7.01±0.21	7.28±0.11	**
Defeathering	7.09±0.13	7.43±0.08	**
Evisceration	6.86±0.15	7.43±0.16	**

After Washing	6.68±0.15	7.36±0.11	**
After Chilling	6.84±0.29	7.30±0.14	**
Employees	6.74±0.18	7.37±0.16	**

N=90

**: Significant difference at P<0.01

±Sd = Standard Deviation



: Fig 1: Comparison of Mean Total Viable Counts of Bacteria (log_{10}CFU/ml) ±Sd at Different Operational Points

At six stages of poultry meat processing (scalding, defeathering, evisceration, after washing, after chilling, and employees) as shown in table 2 and Fig 2 in an automatic poultry slaughter house the high contamination was detected in defeathering stage (mean(log_{10}CFU/ml) 7.09 ± 0.13) low contamination were detected after washing stage

(Mean (log_{10}CFU/ml**)** 6.68 ± 0.15).

Table2: Mean and Standard Deviation at six operation processes in an automatic slaughter house:

CCP	Automatic	Significant
Scalding	7.01±0.21	**
Defeathering	7.09±0.13	**
Evisceration	6.86±0.15	**
After Washing	6.68±0.15	**
After Chilling	6.84±0.29	**
Employees	6.74±0.18	**

±Sd = Standard Deviation

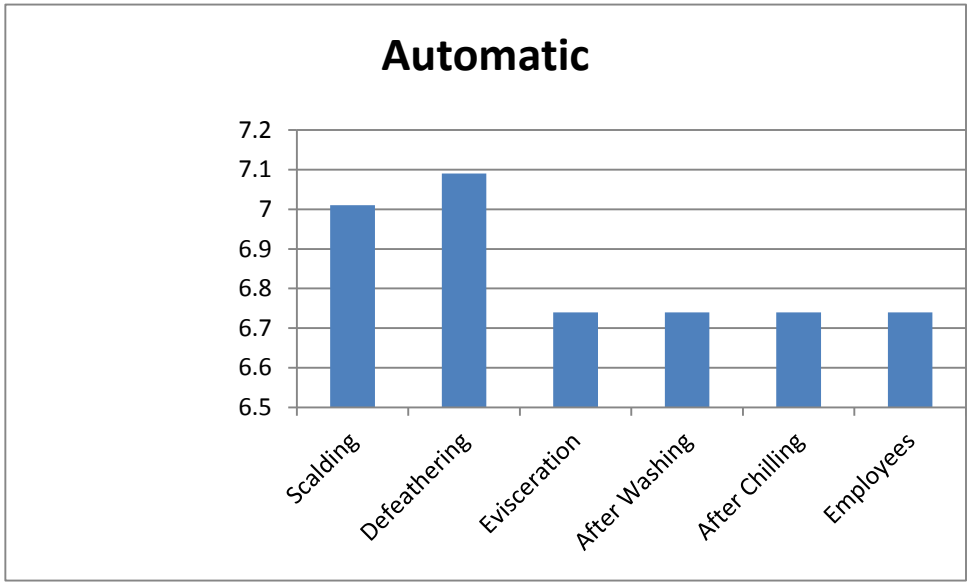


Fig 2: Mean and Standard Deviation at six Operation Processes in an automatic slaughter house

At the six stages of poultry meat processing (scalding, defeathering, evisceration, after washing, after chilling, and employees) as shown in

table 3 (Fig 3) in traditional poultry slaughtering process the high contamination were detected in defeathering stage (mean (log_{10}CFU/ml) 7.43 ± 0.08), low contamination after chilling stage (mean (log_{10}CFU/ml) 7.30 ± 0.14).

Table 3: Mean Total Viable Counts of Bacteria (Log_{10} CFU/ml) ±Sd at Different Operational Point in Traditional Poultry Slaughtering processes.

Control Critical Points CCPs	Traditional	Significant
Scalding	7.28±0.11	**
Defeathering	7.43±0.08	**
Evisceration	7.43±0.16	**
After Washing	7.36±0.11	**
After Chilling	7.30±0.14	**
Employees	7.37±0.16	**

±Sd = Standard Deviation

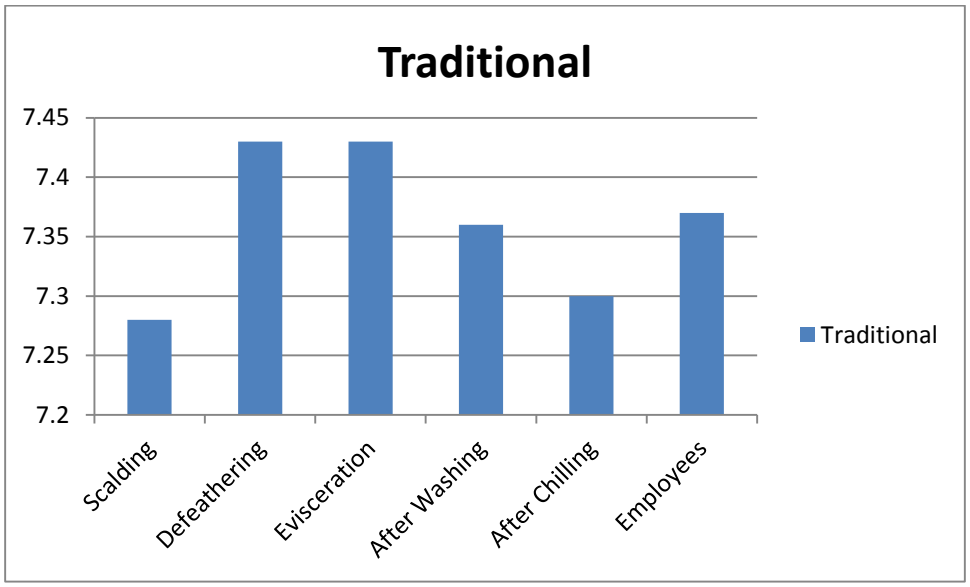


Fig3: Mean total viable counts of bacteria (log_{10} CFU/ml) ±Sd at different operational point in traditional poultry slaughtering processes

The results in table 4(Fig 4) showed the number of isolated bacteria(*Slamonella spp., Escherichia coli and Staphylococcus aureus***)** in different operational points in automatic slaughter house As follows: **Scalding** stage: The number of bacteria isolated as follows 12 (10.4%) samples were positive for *Salmonella spp.,* 3(2.6%) for *Escherichia coli. and* 2(1.8%) samples for *Staphylococcus aureus.*

Defeathering stage: The numbers of bacteria isolated as follows 12(10.4%) samples were positivefor *Salmonella spp., 4*(3.5%) for *Escherichia coli. and* 11(9.6%) for *Staphylococcus aureus*

Evisceration stage: The numbers of bacteria isolated as follows 15 (13%) samples were postive for *Salmonella spp.,* 1(0.9%) for Escherichia *coli and* 4(3.6%) for *Staphylococcus aureus.*

After Washing stage: The numbers of bacteria isolated as follows 15(13%) samples were postive for *Salmonella spp.,* 1(0.9%) for *Escherichia coli. 1*(0.9%) for *Staphylococcus aureus*

After Chilling stage: The numbers of bacteria isolated as follows 15(13%) samples were postive for *Salmonella spp.,* 1(0.9%) for *Escherichia coli. And 1* (0.9%) for *Staphylococcus aureus*

Employees hands stage: The numbers of bacteria isolated as follows 13 (11.3%) samples for *Salmonella spp.,* 2(1.7%) for *Escherichia coli and* 2(1.8%) for Staphylococcus *aureus.*

Table4: Numbers and precentages of (*Salmonella spp. and Escherichia coli, Staphylococcus aureus) isolated* at different process points in An Automatic slaughter house

Critical control points	No. of Samples	*Salmonella.*		*Escherichia coli.*		*Staphylococcus aureus*		Phases %
		Frequency	%	Frequency	%	Frequency	%	
Scaldening	15	12(10.4%)		3(2.6%)		2(1.8%)		17(14.78%)
Defeathering	15	12(10.4%)		4(3.5%)		11(9.6%)		27(23.48%)
Evisceration	15	15(13%)		1(0.9%)		4(3.6%)		20(17.39%)
After Washing	15	15(13%)		1(0.9%)		1(0.9%)		17(14.78%)
After Chilling	15	15(13%)		1(0.9%)		1(0.9%)		17(14.78%)
Employees	15	13(11.3%)		2(1.7%)		2(1.8%)		17(14.78%)

TOTAL	90	82(71.1%)	12(10.5%)	21(18.6%)	115(100%)

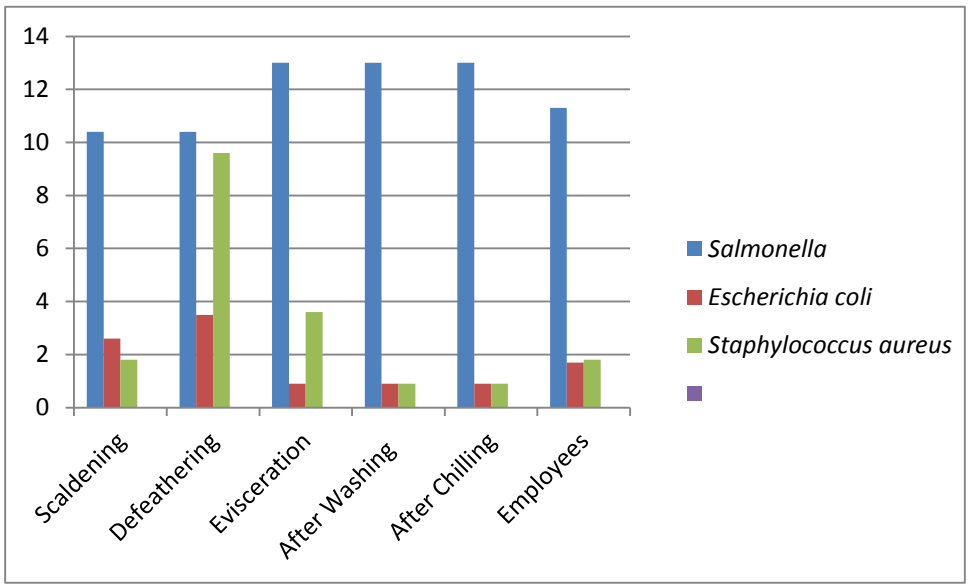


Fig 4: The percentages of *(Salmonella spp., Escherichia coli and Staphylococcus aureus)* isolated at different processing stages in automatic slaughter house

The results in table (5) and (Fig 5) showed the number of isolated bacteria (*Slamonella spp., Escherichia coli and Staphylococcus aureus***)** in different operational points in traditional slaughtering processes as follows:

scaldening stage: The numbers of positive samples were as follows 14(8%) samples were postive for *Salmonella spp.*,6(3.4%) for *Escherichia coli and 9*(5.1%) for *Staphylococcus aureus.*

Defeathering stage: the numbers of positive samples were as follows 12(6.9%) samples were postive for *Salmonella spp.,* 8(4.6%) for *Escherichia coli and* 6(3.4%) for *Staphylococcus aureus.*

Evisceration stage: The numbers of positive samples were as follows 12(6.9%) samples were positive for *Salmonella spp.,* 10(5.7%) for *Escherichia coli and* 8(4.6%) for *Staphylococcus aureus.*

After Washing stage: The numbers of positive samples were as follows 12(6.9%) samples were postive for *Salmonella spp., 3*(1.07%) for *Escherichia coli and* 10 (5.7%) for *Staphylococcus aureus.*

A**fterChilling stage:** The numbers of positive samples were as follows *14*(7.3%) samples were postive for *Salmonella spp.,* 7(4%) for *Escherichia coli and 10*(5.7%) for *Staphylococcus aureus.*

Employees hands stage: The numbers of positive samples were as follows 12 (6.9%) samples were postive for *Salmonella spp., 10* (5.7%) for *Escherichia coli and 12* (6.9%) for *Staphylococcus aureus.*

Table5: Numbers and percentages of positive samples (*Salmonella spp. and Escherichia coli and Staphylococcus aureus)* revealed from the Traditional Slaughtering Processes

Critical control points	No. of Samples	Salmonella.		Escherichia coli.		Staphylococcus aureous		Phases %
		Frequency	%	Frequency	%	Frequency	%	
Scaldening	15	14(8%)		6(3.4%)		9(5.1%)		29(16.57%)
Defeathering	15	12(6.9%)		8(4.6%)		6(3.4%)		26(14.86%)
Evisceration	15	12(6.9%)		10(5.7%)		8(4.6%)		30(17.14%)
After Washing	15	12(6.9%)		3(1.07%)		10(5.7%)		25(14.29%)
After Chilling	15	14(7.3%)		7(4%)		10(5.7%)		31(17.71%)
Employees	15	12(6.9%)		10(5.7%)		12(6.9%)		34(19.43%)
TOTAL	90	76(42.4%)		44(25.01%)		55(32%)		175(100%)

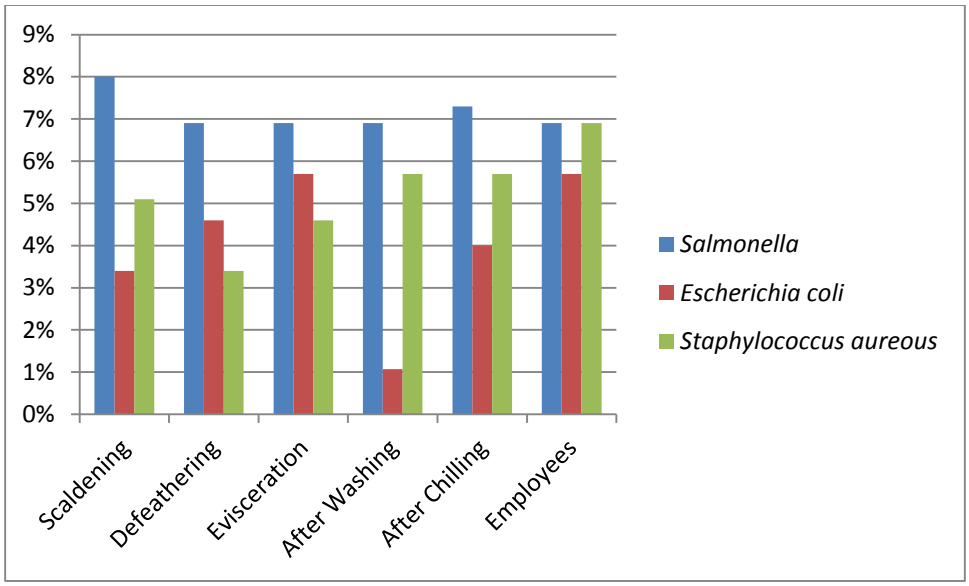


Fig 5: The percentages of *(Salmonella spp., Escherichia coli and Staphylococcus aureus)* isolated at different processing stages in traditional poultry slaughtering processes

A comparison of bacterial contamination between automatic slaughtering processes and traditional poultry slaughtering processes in six Different Operational Points:

At Scalding stages: The results in table 6 (Fig 6) showed high numbers of isolated bacteria in different operational points in traditional slaughtering processes as follows: (mean (log_{10}CFU/ml**)** 7.28±0.11), the numbers of bacteria isolated as follows *Salmonella pp.* 14 (8%), 6(3.4%) *Escherichia coli, and 11 (9.6%) Staphylococcus aureus, in* an Automatic slaughter house showed low contamination(mean(log_{10}CFU/ml**)** 7.01±0.21) and the numbers of bacteria isolated

as follows 12(10.4%) samples were postive for *Salmonella spp.*,3(2.6%) for *Escherichia coli. ,and* 2(1.8%). for *Staphylococcus aureus*

Table 6: A comparison the number of positive samples and their precentages at Scaldening stages in the two type of slaughtering process:

TYPE	CCP	*Salmonella spp.*	*Escherichia coli.*	*Staphylococcus aureus*
Automatic	Scaldening	12(10.4%)	3(2.6%)	2(1.8%)
Traditional	Scaldening	14(8%)	6(3.4%)	9(5.1%)

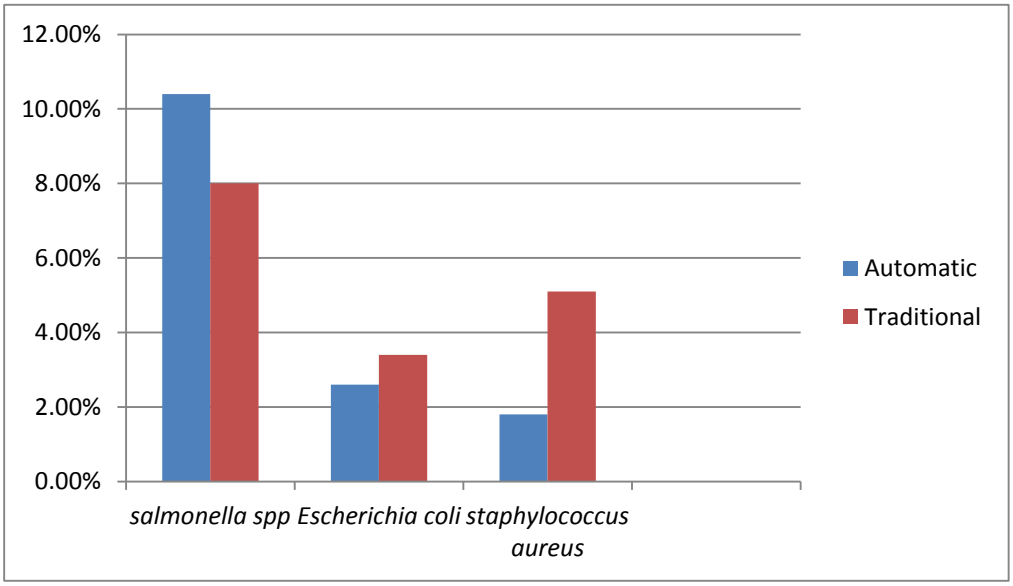


Fig 6: A comparison the percentages of bacterial contaminatiom at Scaldening stages in the two type of slaughtering process

At Defeathering Stages:

Table (7) and (Fig7): Showed high numbers of isolated bacteria in traditional poultry slaughtering processes as follows: Mean (log_{10}CFU/ml**)**7.43±0.08) the numbers of bacteria isolated as follows 12(6.9%) samples were positive for *Salmonella spp.*, 8(4.6%) samples were positive for *Escherichia coli*, and 6(3.4%) samples were positive for *Staphylococcus aureus*, low contamination load in automatic (mean (log_{10}CFU/ml**)** 7.09±0.13) due to low load of bacterial contamination showed the numbers of bacteria isolated as follows *12(10.4%)*samples were positive for *Salmonella spp , 4(3.5%)* samples were positive

for *Escherichia coli, and 11 (9.6%)* samples were positive for *Staphylococcus aureus* .

Table 7: A comparison the number of positive samples and their precentages at Defeathering stages in the two type of slaughtering process

TYPE	CCP	*Salmonella spp;*	*Escherichia coli.*	*Staphylococcus aureus*
Automatic	Defeathering	12(10.4%)	4(3.5%)	11(9.6%)
Traditional	Defeathering	12(6.9%)	8(4.6%)	6(3.4%)

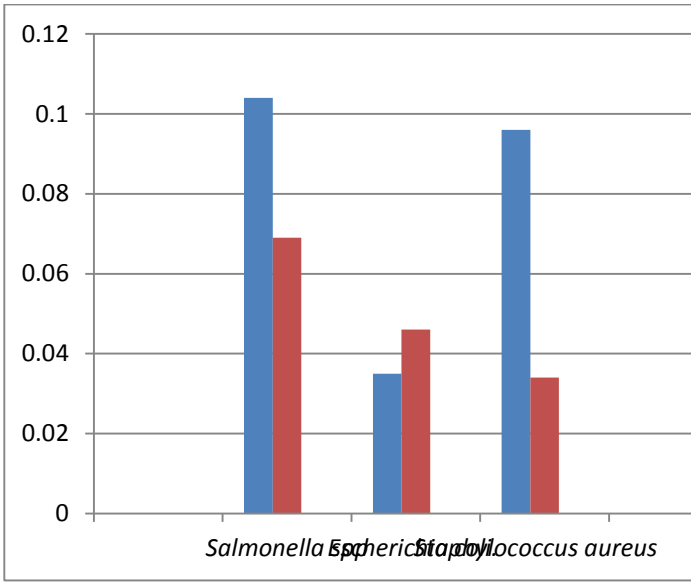


Fig 7: A comparison the percentages of bacterial contaminatiom at Defeathering stages in the two type of slaughtering process

Evisceration Stages In table (8) and (Fig 8): Showed high precentage of isolated bacteria in traditional slaughtering processes (mean(log_{10}CFU/ml**)** 7.43±0.16) and 12 (6.9%) samples were positive for *Salmonella spp.*,10(5.7%) for Escherichia *coli., and* 8(4.6%) for *Staphylococcus aureus* low bacterial contamination (mean(log_{10}CFU/ml**)** 6.86 ±0.15) .In automatic slaughter house, the numbers of

positive samples as follows 15(13%) samples were postive for *Salmonella spp.*,1(0.9%) for *Escherichia coli., and*4(3.6%) for *Staphylococcus aureus*

Table 8: A comparison of positive samples and their precentages in Evisceration in two the type of slaughtering processing .

TYPE	CCP	*Salmonella spp.*	*Escherichia coli.*	*Staphylococcus aureus*
Automatic	Evisceration	15(13%)	1(0.9 %)	4(3.6%)
Traditional	Evisceration	12(6.9 %)	10(5.7%)	8(4.6%)

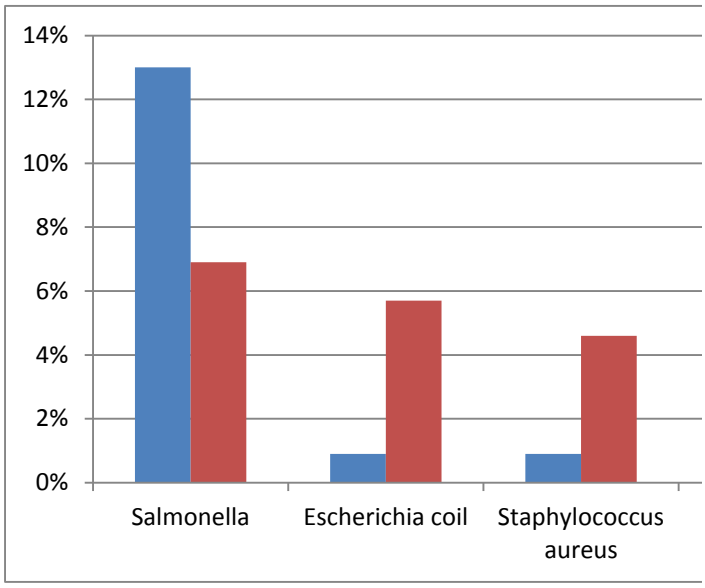


Fig 8: A comparison the percentages of bacterial contaminatiom at Evisceration stages in the two type of slaughtering process

After washing stage:

In table 9 (Fig 9): Showed high numbers of isolated bacteria in traditional slaughtering processes (mean(log_{10}CFU/ml**)** 7.36±0.11.), the numbers of bacteria isolated as follows 12 (6.9%).samples were positive for *Salmonella spp., 3*(1.07%)

for *Escherichia coli., and*10 (5.7%) *for Staphylococcus aureus* , low level of contamination load in an automatic slaughter house showed mean(log_{10}CFU/ml**)** 6.68 ±0.15) the numbers of bacteria isolated as follows 15(13%) samples were positive for *Salmonella spp.* ,1(0.9%) for *Escherichia coli., and* 1(0.9%) for *Staphylococcus aureus.*

Table 9: A comparison of positive samples and their precentages in after washing in two type of slaughtering processing

TYPE	CCP	*Salmonella spp.*	*Escherichia coli.*	*Staphylococcus aureus*
Automatic	Afterwashing	15(13%)	1(0.9%)	1(0.9%)
Traditional	Afterwashing	12(6.9%)	*3*(1.07%)	10(5.7%)

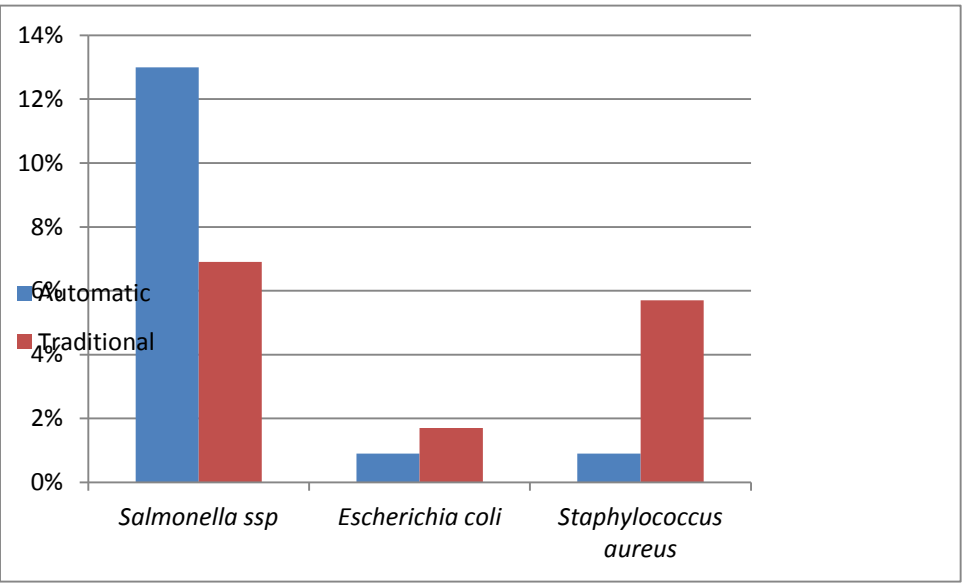


Fig 9: A comparison the percentages of bacterial contamination after washing stages in the two type of slaughtering process

After Chilling stage:

In table 10 (Fig 10): Showed high numbers of isolated bacteria in traditional slaughtering processing (mean(log_{10}CFU/ml**)** 7.30±0.14) the numbers of bacteria isolated as follows 14(7.3%) samples postive for *Salmonella spp.*,7(4%) for *Escherichia coli, and* 10(5.7%) for *Staphylococcus aureus,* low numbers of isolated bacteria in different operational points in an automatic slaughter house*(*mean(log_{10}CFU/ml**)** 6.84±0.29) the numbers of bacteria isolated as follows 15(13%) samples were postive for *Salmonella spp.*, 1(0.9%) for *Escherichia coli and*1(0.9%)for *Staphylococcus aureus*.

Table 10: A comparison between positive sampless and their percentages after chilling in two type of slaughtering processing:

TYPE	CCP	*Salmonella spp.*	*Escherichia coli.*	*Staphylococcus aureus*
Automatic	Afterchilling	15(13%)	1(0.9%)	1(0.9%)

Trad
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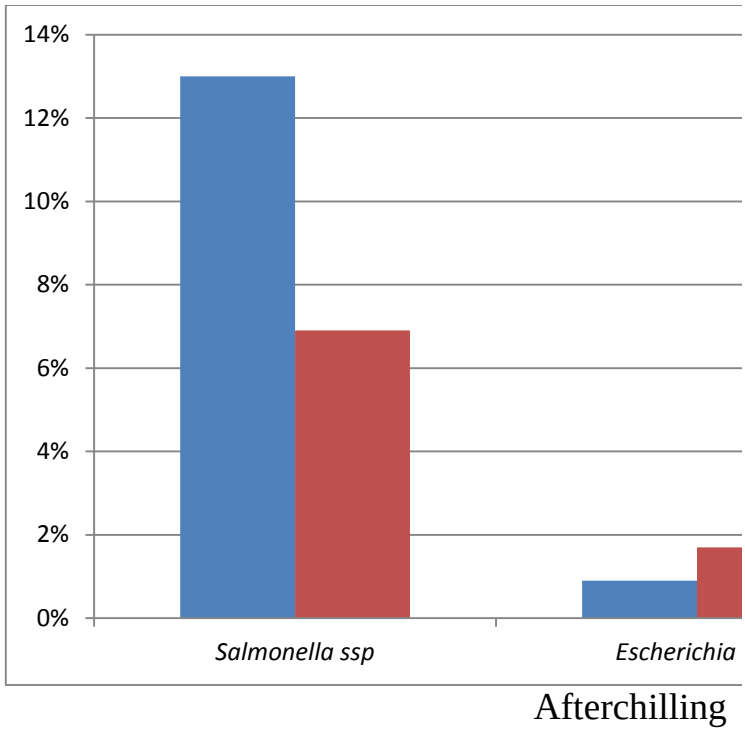
al



14(7.
3%)

7(4%
)

10(5.7%
)

Fig 10: A comparison the percentages of bacterial contaminatiom after chilling stages in the two type of slaughtering process.

Empolyees Hands stage:

In table (11) and (Fig 11): Showed high numbers of isolated bacteria in traditional slaughtering processing (mean(log_{10}CFU/ml**)** 7.37±0.16) the numbers of bacteria isolated as follows 12(6.9%) samples were postive for *Salmonella spp.* ,10 (5.7%)for *Escherichia coli. and* 12(6.9%)for *Staphylococcus aureus* , low numbers of isolated bacteria in different operational points in an automatic slaughter house(mean(log_{10}CFU/ml**)** 6.74±0.18) the numbers of bacteria isolated as follows 13(11.3%) samples were positive for *Salmonella spp.*,2(1.7%) for *Escherichia coli.*, *and* 2(1.8%) for *Staphylococcus aureus*.

Table 11: A comparison between positive samples and their percentages in Embolyees hands in two type of slaughtering processing:

TYPE	CCP	*Salmonella spp.*	*Escherichia coli.*	*Staphylococcus aureus*

Automatic	Employees	13(11.3%)	2(1.7%)	2(1.8%)
Traditional	Employees	12(6.9%)	10 (5.7%)	2(1.8%))

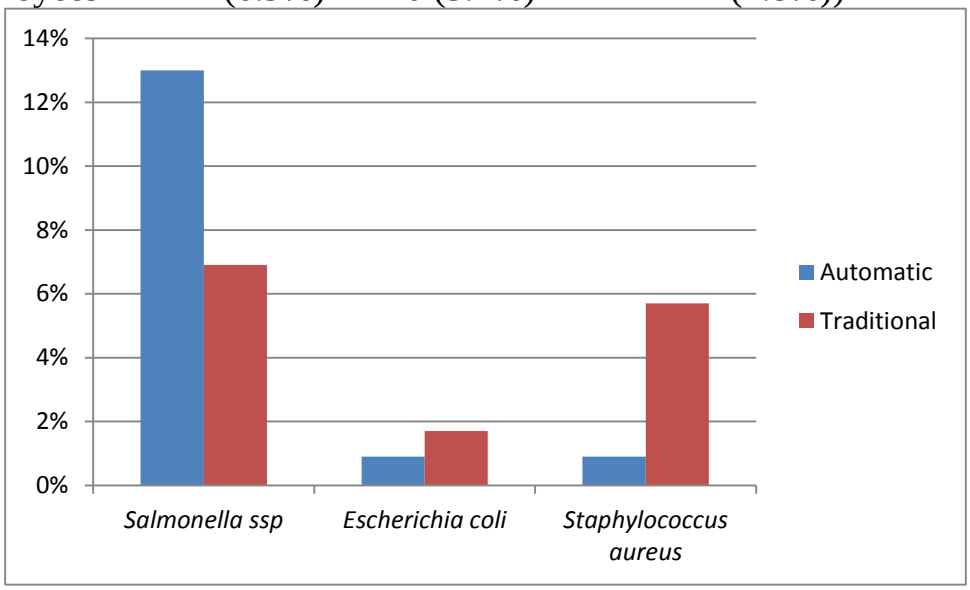


Fig 11: A comparison the percentages of bacterial contaminatiom at Embolyees hands stages in the two type of slaughtering process

Chapter Four

DISCUSSION

Poultry meat contamination with microorganisms which cause deterioration in food quality, and especially those which cause food borne diseases, is a major challenge for poultry industries in many countries that must aim at improving hygiene control during slaughter. In EU member States, principles of good manufacturing practice are used on farms and, for poultry slaughtering and processing, the HACCP system is the most important. Together with preventive measures on poultry farms and the use of modern slaughtering technologies, these systems can guarantee that poultry is produced with minimum microbial contamination and limited incidence of pathogens.*(Perrang et al, (2008).*

In the present results theTotal Viable Count (TVC) revealed that the highest contamination was in the traditional poultry slaughtering processes and lowest

contamination in an automatic poultry slaughter house process. (High significant difference at p-value ($P \leq 0.01$) in all operation processes). (TVC) at scalding point showed high contamination (mean(log_{10}CFU/ml**)** 7.28 ±0.11) in traditional slaughtering process cross-contamination may be due to the birds were lied on the flour in bleeding aera, and low contamination (mean(log_{10}CFU/ml**)** 7.01 ±0.21) in an automatic slaughter house because the birds were suspended at bleeding process.

At Defeathering point the results showed high contamination (mean (log_{10}CFU/ml**)** 7.43 ±0.08) in traditional poultry slaughtering process because the defeathering processed manually , low contamination (mean(log_{10}CFU/ml**)** 7.09 ±0.13) in an automatic slaughter house, because defeathering processed by automatic machine.

Evisceration point showed high contamination (mean(log_{10}CFU/ml**)** 7.43 ±0.16) in traditional poultry slaughtering process, because evisceration processed manully which lead to cross-contamination, low contamination (mean(log_{10}CFU/ml**)** 6.86 ±0.15) in an automatic slaughter house, because evisceration processed by automatic machine.

After washing point showed high contamination (mean (log_{10}CFU/ml**)** 7.36 ±0.11) in Traditional slaughtering processes because washing processed by hands of employees which lead to cross-contamination, low contamination (mean (log_{10}CFU/ml**)** 6.68 ±0.15) in an automatic slaughter house, because washing processed by spary washing automatic machines.

After chilling point showed high contamination (mean (log_{10}CFU/ml**)** 7.30 ±0.14) in traditional slaughtering process, because chilling processed by cold water and ice manually by embolyees in which cross-contamination occur, low contamination (mean (log_{10}CFU/ml**)** 6.84 ±0.29) in an automatic slaughter house, because chilling carcass was processed at suspended chain in chiller room.

Employee's hands showed high contamination due to cross-contamination which occurs because employees did not apply hygienic measures (mean (log_{10}CFU/ml**)** 7.37 ±0.16) in traditional poultry slaughtering process, low contamination (mean (log_{10}CFU/ml**)** 6.74 ±0.18) in an automatic slaughter house because embolyees apply the hygienic measures.

The results showed the number of isolated bacteria (*Salmonella spp., Escherichia coli spp and Staphylococcus aureus***as)** in Different Operational Points in traditional slaughtering processes, scaldening stage

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

Presence of *salmonellae* in chicken meat may be attributed to the healthy state of the living bird which carries *salmonellae,* bad hygienic conditions during slaughtering, cross contamination either from other birds, instruments, machines, workers, scalding tanks, defeathering machines, crop removal, manual evisceration , during slaughter, intestinal contents can spill and contaminte the muscle and organs of the chicken, which is the important source of presence of *Salmonella* in meat and chilling tanks (*Paiao et al., 2013*).This data agree with the present results. 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 poultry meat (Contamination of poultry proparly due to increased used antimicrobials. *Miranda et al, (2008); Adetunji et al, (2011).* Also due to 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 *Bailcy et al, (1987).* This data was in agreement with this study in two types of slaughtering processes.

On studying 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* were encountered *(Abu-Ruwida et.al 1994).* This data was in agreement with the present study.

The presence of *Escherichia coli* in fresh poultry 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 of carcasses may be due to unclean management during the washing, *(Ali 2007)* which was in agreement with this study because there was also contamination in this stage specially in traditional poultry slaughtering process.). The presence of *Escherichia coli* as a contaminant of fresh meat which is reported here has also been reported by several workers. *Ahmed (2004) and Kaboor, (2011)* who suggested faecal contamination as a cause of the large numbers of isolates of *E. coli* recovered. The isolation of bacteria from workers in this study, and the presence of *Salmonella spp., Escherichia coli and Staphylococcus aureus* can be attributed to carcass contaminated with the gastrointestinal contents during processing. An important observation in the present study is that *Salmonella spp.* was isolated from the samples examined. This is in contrast with the result of *Ahmed (2004).*

In the present of study total bacterial viable count (TBCs) showed lower contamination after chilling stage, and highest contamination at defeathering stage. This data is in accordance with the finding of Mead (2004) who reported that substantial decrease in (TBCs 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)* found the Feathers generally may contaminate external surface of the carcasses during early processing stages. (The highest level of viable aerobic bacteria recovered from the samples). Also this is result in agreement with the findings of *Hinton et al, (2000),* who reported that broiler

carcass can be contaminated by bacteria when come in contact with ingest or feces from alminatory tract during grow – out.)

In this study, the mean TBCs obtained from chicken carcasses after slaughter with feathers, after scalding, after defeathering, after evisceration, and scalding were higher than those reported by Göksoy et al, (2004), while the mean TBCs obtained from chicken carcasses after spray wash and after chilling were lower. This difference could be due to environmental and transportation conditions. Moreover, our findings are also higher than the findings of *Kabour (2011)* who reported mean TBCs 7.69±2.6 in legs, 7.49±1.6 in backs and 8.38±2.1 in breasts after defeathering. But the mean TBCs obtained from chicken carcasses after spray wash and after chilling and packing were lower than those reported. It is obvious that the variability of microbial counts indicates the need for use of prerequisite programs. Our present study revealed that at defeathering stage low contamination load was detected in automatic poultry slaughter house (mean(log_{10}CFU/ml**)** 7.09±0.13) due to low bacterial contamination as shown as the isolated bacteria(10.4%) samples were positive for *Salmonella spp.* ,4(3.5%) samples were positive for *Escherichia coli,* and 11(9.6%) samples were positive for *Staphylococcus aureus.* The manual defeathering processed in traditional poultry slaughtering processing was high contaminated with the isolated bacteria *12(6.9%)* samples were positive for *Salmonella spp., 8(4.6%) for Escherichia coli, and 6(3.4%) for Staphylococcus aureus*.

As reported by *Nather et al. (2009),* the evisceration process is very conducive to increased contamination in slaughter houses, since the exposure of the bird internal organs may result in contamination of the carcass. Reinforcing those findings, *Soares et al. (2002)* observed an increase in the number of *Enterobacteriaceae* in the carcass after evisceration comparing to other processing stages. Such conclusions corroborate the view of *Soares et al, (2002)* when reporting that one of the biggest problems in poultry processing is carcass contamination by fecal matter during evisceration.x Broilers arriving to the poultry slaughter house for processing are generally highly contaminated with bacteria, especially with potential human pathogenic bacteria, such as Coliform and *Salmonella* . Our present study revealed that evisceration stage high bacterial contamination (mean (log_{10}CFU/ml**)** 7.43 ±0.16) in traditional poultry slaughtering process and low bacterial contamination

at Evisceration stage (mean (log_{10}CFU/ml**)** 6.86 ±0.15) .In automatic slaughter house. Low bacterial contamination as shown the numbers of positive samples were as follows 15(13%) for *Salmonella spp.,* 1(0.9%) for *Escherichia coli., and* 1(0.9%) for *Staphylococcus aureus* and high contamination in evisceration stage at traditional slaughtering processing due to found bacteria as shown the numbers of positive samples were as follows 12 (6.9 %) for *Salmonella spp.,* 10(5.7%) for *Escherichia coli and* 8(4.6%) for *Staphylococcus aureus,* because cross-contamination by intestinal contents which included bacteria.

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 is agree with (Ali 2007). High bacterial contamination in washing stage in a traditional slaughtering process (mean7.36±0.11) with 12(6.9%) samples were positive for *Salmonella spp., 3*(1.07%) for *Escherichia coli.,* and10 (5.7%) for *Staphylococcus aureus* because its washed in one tank water, low level of contamination load for washing stage in an automatic slaughter house showed positive samples were as follows 15(13%) for *Salmonella spp.,* 1(0.9%) for *Escherichia coli., and* 1(0.9%) for *Staphylococcus aureus,* because it's washed by spraying wash. Reported by *Barbalho et al. (2005),* who describe that the contamination of carcasses occurs mainly during or after the evisceration and chilling stages, and also evaluated a poultry slaughterhouse in Bahia, Brazil, and found contamination in 14.3% of the carcasses already packed.This agrees with our present study.

Our present study there was high bacterial contamination at the chilling stage in a traditional slaughtering processing (mean(log_{10}CFU/ml**)** 7.30±0.14) with the numbers of isolated bacteria 14(7.3%) samples were postive for *Salmonella spp.,* 7(4%) for *Escherichia coli, and* 10(5.7%) for *Staphylococcus aureus,* because the chilling in cold water due to cross-contamination, low level of bacterial contamination chilling stage due to carcasses found in chiller room in an automatic

slaughter house *(mean* (log_{10}CFU/ml**)** 6.84±0.29)with the numbers of isolated bacteria 15(13%) samples were positive for *Salmonella spp.,* 1(0.9%) for *Escherichia coli, and*1 (0.9%) for *Staphylococcus aureus*, because the chill process in chiller room. Also *Göksoy et al. (200*4) demonstrated the presence of *Staphylococci species* at different stages of processing.

This finding is similar to our result but contrary to the findings of Kabour (2011) who did not detect any *Staphylococci species* in his study. Industries must implement this food safety program to serve both internal and external market *(Jimenez et al, 2002; Mead, 2004).* Sudanese abattoirs may reflect the hygienic status of chicken meat production in the developing countries.

The automation of slaughter plant led to the reduction of contamination, a fact that was shown from the microbiological analysis results, which indicated a reduction of the micro-organisms under study (total bacterial count, *coli forms* and *Escherichia coli*) in the various stages of slaughter and standardization procedure. 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.

The reduction of the microbial contamination in this study is in agreement with *Rahkio and Korkeala (1996),* who said that the enforcement of hygienic practice such as regular disinfection of working tools and workers' hands is important in reducing the microbiological contamination of carcasses. *John et al. (2000)* reported that the reduction of bacterial contamination during slaughtering after using a degree of sanitation. Another study by *Jeffery et al. (2003)* revealed that the workers' hands and the equipments were the sources of meat contamination; these results are in accordance with the present results. The elimination of contamination sources by practicing good sanitary measures will reduce the occurrence of microorganisms. Appropriate methods should be applied during slaughtering operations, using adequate water and disinfection. Sudan is a tropical country, with ambient temperatures conducive for the growth of microorganisms, which can rapidly render meat unsafe for human consumption. The levels of microbial contamination in

Sudanese abattoirs may reflect the hygienic status of poultry meat production in the developing world. Qualitative bacterial examination of carcass samples in this study revealed results that are in agreement with the findings of *Ahmed, (2004) and Kaboor, (2011).*

The present study demonstrates the degree of the microbial contamination during processing of broilers carcasses. The results also indicate that the viable count for microorganisms causing public hazards is appropriate for analysis. Therefore, application of hygienic measurements appears to be important to reduce the contamination of bacteria in abattoirs. So the implementation of Hazard Analysis and Critical Control Points (HACCP) in poultry industry is extremely important, because it involves the constant monitoring of all steps of the process.

Although the present results have shown a lower contamination rate than that seen in other studies, the risk to consumer health as well as the economic losses associated with these microorganisms make their continuous monitoring a relevant action. The results provide support for the development of strategies aiming the industrial control of the bacteria analyzed. They stress the need to effectively implement the Good Manufacturing Practices (GMP) in poultry industries by keeping, in particular, a meticulous control of the evisceration, chilling and operational hygiene processes as a measure to reduce the contamination levels caused by the microorganisms investigated.

It is quite difficult to define a best model for food-safety practices applicable to the developing world as a whole. More country-specific data on risk factors throughout the vertical chain are needed. The political environment, the state of infrastructure and so forth should also be carefully assessed before policies are formulated.

Surveillance and data collection systems are often lacking or not functional, meaning that reliable data about risk factors are unavailable. Restructuring or establishing food- safety services may require substantial education of veterinary and the health inspectors at all levels. A market-driven approach could be a way to achieve success in food safety, but this would need interest and large investments

from the industry. There would definitely be difficulties in implementing a thorough control system, because of the existence of the vast informal sector in which poultry.

CONCLUSION & RECOMMENDATION

Conclusion:

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

1/ There is contamination in an automatic poultry slaughterhouse in Khartoum State, and traditional slaughtering processes

2/ *Salmonella species and Escherichia Coli and Staphylococcus aureus* were isolated from poultry meat at all stages of processing.

3/ the highest contamination were shown at all stages of Traditional slaughtering processes. And lowest contamination at an automatic slaughter house processes.

4/ Most of Automatic Poultry Slaughter house and Traditional slaughtering processes are not Applying HACCP System.

Recomendation

DATA concerning the control of microbiological hazards from/upon the poultry in all stages of poultry processing, storage, transport and retail of poultry. Need Application of the following:

1/ Current standards for microbiological control to ensure quality assurance and safety of poultry slaughter process.

2/ Risk management and processing of poultry meat getting

3/Implementation of good hygiene in poultry processing; Training should be given to workers in the abattoir, especially for those who are assigned in poultry meat carcass process about the contamination sources and hygienic conditions to maintain the quality of the poultry meat carcasses

4/ Sanitation in the poultry breeding farms and poultry processing plants;

5/ Application of HACCP in poultry processing, based on the use of multi - functional strategies (sanitizers &modern disinfections techniques).to reduce bacterial contamination.

Consider personnel hygiene, handsgloves, masks, head cover, contaminated equipment cross-contamination from raw material as plucked fingers during defeathering stage. , valves or plates

6/ Cooking at high temperatures of 100c$^{\circ}$ will help to eliminate pathogens before consumption.

7/ Application of hygienic measurements appears to be important to reduce the contamination of bacteria in abattoirs. So the implementation of Hazard Analysis and Critical Control Points (HACCP) in poultry industry is extremely important, because it involves the constant monitoring of all steps of the process.

Appendix (1): Total Bacterial Counts at Six Critical Points in An automatic slaughter house:

NO.	Time of test	Phase A Average cpu/ml		Phase B Average cpu/ml		Phase C Average cpu/ml	
		Stage1	Stage2	Stage1	Stage2	Stage1	Stage2
1	Scalding	10×10^4	9×10^5	8×10^4	6×10^5	8×10^4	6×10^5
2		5×10^4	4×10^5	4×10^4	4×10^5	4×10^4	7×10^5
3		4×10^4	7×10^5	5×10^4	5×10^5	5×10^4	5×10^5
4		9×10^4	4×10^5	3×10^4	3×10^5	3×10^4	3×10^5
5		9×10^4	11×10^5	3×10^4	2×10^5	2×10^4	2×10^5
6	Defeathering	8×10^4	7×10^5	9×10^4	7×10^5	9×10^4	7×10^5
7		5×10^4	5×10^5	10×10^4	7×10^5	10×10^4	7×10^5
8		6×10^4	5×10^5	7×10^4	7×10^5	7×10^4	7×10^5
9		9×10^4	8×10^5	6×10^4	4×10^5	6×10^4	4×10^5
10		4×10^4	5×10^5	3×10^4	3×10^5	2×10^4	4×10^5
11	Evisceration	3×10^4	4×10^5	4×10^4	3×10^5	3×10^4	4×10^5
12		8×10^4	6×10^5	4×10^4	4×10^5	4×10^4	4×10^5
13		4×10^4	4×10^5	5×10^4	3×10^5	5×10^4	3×10^5
14		5×10^4	5×10^5	3×10^4	2×10^5	2×10^4	3×10^5
15		4×10^4	4×10^5	1×10^4	2×10^5	2×10^4	2×10^5
16	After wash	6×10^4	4×10^5	3×10^4	2×10^5	3×10^4	2×10^5
17		5×10^4	4×10^5	2×10^4	2×10^5	2×10^4	2×10^5
18		2×10^4	3×10^5	2×10^4	2×10^5	2×10^4	2×10^5
19		4×10^4	2×10^5	4×10^4	4×10^5	4×10^4	4×10^5
20		2×10^4	2×10^5	2×10^4	1×10^5	1×10^4	2×10^5
21	After Chill	4×10^4	3×10^5	2×10^4	2×10^5	2×10^4	3×10^5
22		4×10^4	3×10^5	3×10^4	3×10^5	3×10^4	3×10^5
23		2×10^4	2×10^5	3×10^4	2×10^5	3×10^4	2×10^5
24		2×10^4	2×10^5	2×10^4	3×10^5	2×10^4	3×10^5
25		4×10^4	4×10^5	6×10^4	2×10^5	6×10^4	4×10^5
26	Employees	5×10^4	3×10^5	5×10^4	3×10^5	5×10^4	3×10^5
27		5×10^4	3×10^5	3×10^4	3×10^5	3×10^4	5×10^5
28		3×10^4	3×10^5	3×10^4	2×10^5	2×10^4	3×10^5
29		3×10^4	2×10^5	2×10^4	1×10^5	2×10^4	1×10^5
30		2×10^4	4×10^5	2×10^4	2×10^5	2×10^4	2×10^5

Appendix (2): Total Bacterial Counts at Six Critical Points in Traditional slaughtering process:

NO.	Time of test	Phase A Average cpu/ml		Phase B Average cpu/ml		Phase C Average cpu/ml	
		Stage1	Stage2	Stage1	Stage2	Stage1	Stage2
1	Scalding	13×10^4	9×10^5	19×10^4	10×10^5	18×10^4	15×10^5
2		11×10^4	8×10^5	17×10^4	13×10^5	15×10^4	9×10^5
3		10×10^4	7×10^5	13×10^4	8×10^5	12×10^4	10×10^5
4		9×10^4	6×10^5	8×10^4	7×10^5	12×10^4	8×10^5
5		9×10^4	7×10^5	8×10^4	11×10^5	8×10^4	6×10^5
6	Defeathering	12×10^4	10×10^5	15×10^4	14×10^5	14×10^4	14×10^5
7		14×10^4	11×10^5	14×10^4	9×10^5	11×10^4	11×10^5
8		18×10^4	14×10^5	20×10^4	17×10^5	12×10^4	11×10^5
9		22×10^4	13×10^5	13×10^4	10×10^5	20×10^4	14×10^5
10		13×10^4	9×10^5	16×10^4	13×10^5	17×10^4	14×10^5
11	Evisceration	15×10^4	12×10^5	22×10^4	19×10^5	11×10^4	9×10^5
12		22×10^4	19×10^5	20×10^4	13×10^5	10×10^4	11×10^5
13		21×10^4	16×10^5	12×10^4	10×10^5	8×10^4	7×10^5
14		17×10^4	14×10^5	14×10^4	11×10^5	7×10^4	7×10^5
15		22×10^4	17×10^5	8×10^4	7×10^5	23×10^4	20×10^5
16	After wash	20×10^4	10×10^5	11×10^4	20×10^5	12×10^4	9×10^5
17		10×10^4	10×10^5	11×10^4	8×10^5	22×10^4	14×10^5
18		14×10^4	10×10^5	12×10^4	9×10^5	15×10^4	9×10^5
19		10×10^4	9×10^5	11×10^4	12×10^5	13×10^4	13×10^5
20		9×10^4	7×10^5	10×10^4	8×10^5	10×10^4	9×10^5
21	After Chill	10×10^4	7×10^5	11×10^4	8×10^5	14×10^4	13×10^5
22		10×10^4	8×10^5	9×10^4	6×10^5	19×10^4	18×10^5
23		11×10^4	8×10^5	8×10^4	8×10^5	9×10^4	7×10^5
24		10×10^4	7×10^5	10×10^4	10×10^5	10×10^4	8×10^5
25		13×10^4	9×10^5	9×10^4	7×10^5	18×10^4	17×10^5
26	Employees	20×10^4	18×10^5	14×10^4	11×10^5	11×10^4	7×10^5
27		16×10^4	8×10^5	17×10^4	13×10^5	21×10^4	15×10^5
28		20×10^4	11×10^5	20×10^4	9×10^5	9×10^4	6×10^5
29		21×10^4	12×10^5	9×10^4	7×10^5	14×10^4	9×10^5
30		22×10^4	19×10^5	9×10^4	6×10^5	13×10^4	12×10^5

Appendix 3:

Bacteria	*Salmonella spp.*	*Escherichia coli*	*Staphylococcus aureus*
Media	1.MacConkey Agar 2.Dexoycholate Agar	1.MacConkey Agar 2.Dexoycholate Agar	1.Mannitol Salt Agar
Color	1.Pale yellow or colorless 2.Slight Smaller after 48hs. Colonies developed black centre	Pink color	1.opaque with yellow zone

Biochemical test of *Escherichia Coli and Salmonella and Staphylococcus* Appendix 4*:*

Tests	Salmonella	Escherichia coli	Staphylococcus
Indole production	-	+	-
Methyl Red(MR)	+	+	+
Voges-Prokaur(VP)	-	-	+
Urease test	-	-	+
H_2S Production	+	-	
Phenylalanine deaminalinase test	-+	-	
Gelatin liquefaction test	+	-	
Melonate utilization	+	-	
Acid Gas Form			
Glucose	+	+	+
Mannitol	+	+	+
Maltose	+	+	+
Lactose	-	+	+
Sucrose	-	+	+
	Decarb0xylation Lysine + Ornithine + Arginine +		**Nitrate Reduction +**

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