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A study of Microbial Contamination and drug sensitivity in Broilers in Modern Slaughter House in Khartoum State

دراسة التلوث الجرثومي واختبار حساسية الدواء للدجاج اللاحم في مجزر آلي حديث بولاية الخرطوم

A Thesis Submitted to the College of Graduate Studies in the Fulfillment of the Requirement of Attaining Master of Science in Veterinary Preventive Medicine and Public Health

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Dedication

To my parents, sisters, brothers and my sincerely wife and daughter

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Firstly, praise to Almighty Allah for giving me the strength and stamina to finish this work.

With a great touch of pleasure and gratitude, I would like to express thanks to my supervisor, Professor Mohamed Abdelsalam Abdalla for his advice, direction and continuous interest and constructive criticism in reviewing the dissertation.

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Abstract

This study was planned to investigate presence of bacterial contamination of broilers breast, back, leg and neck during slaughtering process. A total of 154 swab samples were taken after hanging in slaughter area, after bleeding, after scalding, after defeathering, after evisceration, after washing, after chilling, from slaughtering knives, scalding water, packing machine and hands of the workers. Total viable counts (TVCs) and isolation and identification of bacteria were used. All isolates were tested for some antibiotics (gentamyin, amoxicillin, ciprofloxacin, vancomycin, erythromycin, penicillin, chloranphenicol). The results revealed no statistical difference (P >0.05) between sites of the carcasses and critical points during slaughtering process. The isolated bacteria were E.coli, Salmonella spp. , Proteous spp. , Pseudomonas spp. and Staphylococcus spp. The poultry carcasses can be contaminated at any stage of slaughtering process. Therefore, hygienic measures must be managed to safe broiler meat for human consumption.

ملخص الدراســــــــــــة

هذه الدراسة هدفت الي معرفة التلوث الجرثومى فى العنق والارجل و الصدر و الظهر فى الدجاج اللاحم التلوث الجرثومي بعد الذبح و أثناء التصنيع ، علي ذبائح الفراريج ، من خلال تطبيق نظام تحليل المخاطر ، عند نقاط التحكم الحرجة ،فى مجازر حديثة لذبح الدواجن، في ولاية الخرطوم.

من احدي عشر نقطة تحكم حرجة) CCPs(على خط تصنيع الفراريج، تم جمع 451 عينة وتم فحصها لتحديد التلوث الجرثومي وتم عزل البكتريا ومن ثم اختبرت حساسيتها لبعض المضادات الحيوية)جينتا مايسين, اموكسسلين , سيبروفلوكسسين ,فانكومايسين , اريثرو مايسين , البنسلين, الكلورافيناكول(.

وأظهرت النتائج التي تم الحصول عليها في النقاط الحرجة الاحدى عشر) CCPs(فى العنق ، الارجل ، الظهر ، والصدر عدم اختلافات بين هذه النقاط والمناطق) p<5...(.

البكتريا التي وجدت في كل نقطة حرجة أعطى دليلا على أن مرحلة ما بعد الذبح يشكل خطرا علي جودة وسلامة لحوم الدواجن ، وفي هذه الدراسة وجدنا تلوث مقبول يحتوي علي خمس انوا من البكتريا السلامونيا والايكولاي والاستاف وسودوموناس والبروتيس ، بحيث يمكن ان تؤثر في جودة وسلامة لحوم الدواجن المنتجة تجاريا بولاية الخرطوم اذا لم يتبع الحلول الامنة والفعالة للتقليل منها.

Introduction

Production and consumption of poultry meat and poultry meat products show an upward trend. This, of course, requires adequate control and inspection both during poultry rearing and in slaughterhouses, processing plants and shops. Consumers are also a link in the chain of food-borne human diseases, because of the way they store and cook poultry meat and meat products. Special attention in poultry meat production is paid to the fact that live animals are hosts to a large number of different microorganisms residing on their skin, feathers or in the alimentary tract. During slaughter most of these microorganisms are eliminated, but subsequent contamination is possible at any stage of the production process, from feather plucking, evisceration, and washing to storage by cooling or freezing. Microorganisms from the environment, equipment and operators' hands can contaminate meat (Mead, 1989; Živkovic, 2001). During the process, the microflora changes from, in general, Gram-positive rods and micrococci to, most frequently, Gramnegative bacteria in final products, including enterobacteria, *Pseudomonas* spp., etc. Industrial poultry slaughterhouses have a technological process, the individual stages of which are not in conformity with modern principles of hygienic meat production and processing (Kozačinski *et al*., 2006).

Food safety was identified as a high priority area in the 2001–2005 World Organisation for Animal Health (OIE) Strategic Plan. Member countries of the OIE considered that the organisation should be more active in issues of public health and consumer protection and that this should include more involvement in the area of diseases or pathogens transmissible through food (Droppers, 2006). Meat and meat products are of particular importance regarding foodborne illnesses. Foodborne

pathogens can be introduced to foods during processing, storage and preparation from infected humans who handle the food or by cross contamination from some other raw agricultural products (Hedberg *et al*., 1994).

Although it is impossible to ensure the complete absence of pathogens from broilers, the risk of foodborne disease can be reduced substantially by minimizing their numbers. Mead (1989) summarized the reasons why controlling microorganisms in poultry processing is difficult , the rapid rate of production keeps the birds in close proximity throughout processing, limitations in the design of processing equipment, including that used in scalding, defeathering, and evisceration, the difficulty of washing the abdominal cavity effectively after evisceration when the carcass remains whole, retention of water by skin, which tends to entrap bacteria in the crevices and feather follicles (Notermans and Kampelmacher, 1974; McMeekin and Thomas, 1979).

Hazard Analysis Critical Control Points (HACCP) is a well-accepted systematic program for identification and control of microbiological hazards associated with poultry processing and has been applied to the poultry industry to improve microbiological quality of broiler carcasses and reduce microbiological hazards from farm to consumption (Unnevehr and Jensen, 1996; McNamara, 1997).

The objectives of this study was:

1. To identify the most contaminated site of the surface of the carcass.
2. To determine bacterial number (total viable counts) on broiler meat.
3. To isolate and identify bacteria at different check points.
4. To determine the effective antibiotic to the isolated bacteria.

Chapter One
Literature Review

1.1 History of HACCP

The Pillsbury Company first developed the concept of HACCP in the early1960's, this firm worked cooperatively with NASA to develop this new system to ensure safety of the food consumed by the astronauts. At that time, most safety systems were based on product testing. For this concept to be fully effective, companies must test 100% of their product. Since most testing is destructive, this approach would not be able because the entire product would be required (Mortimore and Wallace, 2000). At the 1971 National Conference on Food Protection, the HACCP system was first presented. This new approach to food safety gained interest among food processors 3 and was used as the basis for regulations regarding low-acid and acidified foods. Furthermore, the FDA even began using HACCP for investigation activities. However, after the initial excitement of the new system, interest in HACCP began to fade. According to Stevenson (1990), only a few large companies continued to apply HACCP. During the 1980s, some of the government protection agencies asked NAS/NRC (National Academy of Sciences/National Research Council) to form a committee that would generate some general principles for the application of microbial criteria in foods.This committee proposed the implementation of HACCP in food protection programs. In addition, they suggested that the food industry receive the proper training regarding the HACCP concept (Stevenson, 1990). Many food industries have implemented HACCP since its inception. Some have done so voluntarily, whereas others have been mandated. Industries currently mandated are Seafood (since 1997) and Juices (effective in 2002). The meat and poultry industry fell under the HACCP

mandate in 1998(large plants). Small and very small plants, Followed in 1999 and 2000, respectively. The smaller plants were given more time to develop their HACCP plans due to fewer resources and personnel compared to larger plants (Bowers, 1998). The canned food industries do not have a mandatory HACCP requirement, but one is highly recommended. The major reason that some canning companies have implemented HACCP is to control *Clostridium botulinum* (Food Safety and Inspection Service, 2000).

1.2 History of Poultry Industry in the Sudan

In Sudan, Agriculture is the largest economic sector, in terms of contribution to the GDP and is the largest employer. Even if the agricultural sector has been demolished due to the oil industry, Sudan is still dependent on its agriculture since it stands for 40 percent of the GDP (Freiji, 2008).

The world's poultry production provides the human population with two main products:
table eggs and broiler meat. Multinational agribusinesses3 have taken over the industry in the western world and are, step by step, penetrating the markets in developing countries as well. Also, in Sudan, modern automatic control systems and record keeping are becoming the normal rather than the exception. As the economy is growing, urbanization from the rural areas towards cities is making people dependent on agro-industries which provide easy access to food (Freiji, 2008). History of the poultry industries in the Sudan began in 1926, by enter a group of Yandotte Chicken from British, followed establishment of the central poultry farm in Khartoum Bahri in 1951 this was starting point of government investment in the field of poultry farming. In 1958 was published a first version of a book on behalf of poultry (poultry farming in the Sudan) to author. Makelmenjeri. Later in

1963 the American Aid Programme established Kuku Poultry Farm. Breeds such as White Leghorn, Fayoumi, Rhode Island Red, New Hampshire and Light Sus

sex were introduced into the Sudan (El-lssawi, 1977; Nagla, 1998). During the period from 2001 - 2005 a significant increase in the number of farms, because of growing demand and an improvement in selling prices, especially after the increase in population steady in the state of Khartoum. According to field survey in 2009 the production of broilers was 17.3 million chicks, and the poultry factories in Khartoum state, were about 10 factories of the poultry broilers production with capacity of 25000 tons / hour (Nagla, 1998). The major source of chicken meat and egg in the Sudan was produced from a population estimated in 1975-1976 to be about 22 million birds yielding 1.3 million kilograms of meat (El-lssawi, 1977). He also stated that the Government was aware of the importance of developing poultry in the Sudan and hence laid down in 1976 the promotion of Agriculture Investment Act. Because of this Act a joint Venture the Sudanese Alkwietia Poultry Company was established which at present called the African Poultry Company. The production was five million birds and 40.000 tons of poultry feed. The Arabia Poultry Company started operation in 1984 for Poultry meat and egg self-satisfaction. It produced million eggs and two million kilograms of poultry meat yearly. The broilers produced were inspected pre-slaughtering. A broiler was the trade name used for a young male or female chicken about 1.5 kilograms and 8 weeks of age (Gracey, 1981). With the establishment of the broilers processing in Khartoum State, the inspection of poultry meat has been covered in the Meat Inspection Act of the 1974 of the Sudan Laws (Nagla, 1998).

1.3 Introduction of HACCP

The Hazard Analysis Critical Control Point (HACCP) system is a scientific approach to process control. It is designed to prevent the occurrence of problems by assuring that controls are applied at any point in a food production system where hazardous or critical situation could occur. Hazards include biological, chemical, or physical contamination of food products. The Food Safety and Inspection Service (FSIS) published a final rule in July 1996 mandating that HACCP be implemented as the system of process control in all inspected meat and poultry plants. As part of its efforts to assist establishments in the preparation of plant-specific HACCP plans, FSIS determined that a generic model for each process defined in the regulation would bemade available for use on a voluntary basis by inspected establishments (USDA, 1999).

1.4 Principles of HACCP

According to Snyder (1991), the seven principles that now make up a HACCP plan and these are:

1.4.1 Principle 1

The first principle involves conducting a hazard analysis, which involves assessing certain risks involved in production of a product. “Hazard Analysis is defined as ‘the process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan’” (Mayes, 1999).

1.4.2 Principle 2

When the hazard analysis is complete, the HACCP team must go over the flow diagram and decide which steps are critical control points (CCPs). A CCP can be a point in the process where a significant hazard can be eliminated or reduced to an acceptable level. A CCP is also a point where loss of control will lead to a significant hazard. The differance from a control point (CP) in that a loss of control at a CP will not lead to a significant

hazard. Two examples of common CCPs are cooking and chilling, because these steps are designed to reduce the occurrence of a hazard. CCPs require a lot of careful development and extra documentation and that is why they should be limited to only those that are truly critical (Weddig, 1999).

1.4.3 Principle 3

Once the CCPs are determined, critical limits are required for each step that is a CCP. A critical limit is a maximum or minimum value to which a specific parameter must be controlled at each CCP. Common critical limits are temperature, time, moisture, pH and salt concentration. Critical limits are rarely a range of values. Each limit should have some sort of basis whether that is FSIS regulations, FDA action levels, or any other scientific literature (Food Safety and Inspection Service, 1996).

1.4.4 Principle 4

The next step is to monitor each CCP and critical limit. Monitoring of each critical limit is very important because it helps to ensure that the CCPs are complying and the critical limits are not exceeded (Sohrab, 1999).

1.4.5 Principle 5

If there is a deviation from the set standards of a critical limit, corrective actions must be taken (Snyder, 1991). Corrective actions are procedures carried out when a loss of control has occurred at a CCP. Sperber (1991) suggested that all corrective actions as well as- responsibilities should be clearly outlined before HACCP is implemented. All records and corrective actions should be documented to prove that corrective actions are being conducted (Sohrab, 1999).

1.4.6 Principle 6

The next principle that must be addressed is verification. Verification is the application of methods, procedures and tests to determine the company's compliance with the HACCP plan (Mayes, 1999).

Verification covers all internal daily activities with regards to HACCP (Lupin, 2000).

1.4.7 Principle 7

The seventh principle of HACCP is to establish adequate record keeping procedures. Without records, there is no proof that a plant is doing what their HACCP plan indicates. According to Sohrab (1999), the purpose of recording keeping is to show that the HACCP plan is compliant with the documented system. Records are useful in providing a basis for trends and for system at c improvement of the process over time (Snyder, 1991).

1.5 HACCP benefits

According to studies by Aramounii *et al*., (1996) since HACCP implementation, there has been reduced microbial contamination on equipment surfaces tested in meat plants.

Microbial results on meat grinders, knives and plastic lugs were all reduced a least 1 log CFU/cm2. HACCP has also improved the regulatory aspect of food safety by offering an opportunity for food control authorities to revisit their method of inspection. The HACCP system has increased the collaboration among scientists, which will essentially strengthen the abilities of food safety authorities in producing safe food (Motarjemi and Kaferstein, 1999). According to Motarjemi and Kaferstein (1999) if a HACCP plan is based on sound science, it will prevent many outbreaks by improving hygienic quality of foods.

1.6 Purpose of HACCP

The HACCP program serves several purposes. The main objective of HACCP is to produce a safe product. HACCP is a safety program, not a

quality program. Metal fragments, microorganisms that cause illness, and harmful chemicals are examples of some of the hazards that HACCP will attempt to reduce or eliminate (Swanson and Anderson, 2000). There will never be a process that is safe, but there must always be a constant effort to achieve zero defects (Snyder, 1991). Another function of HACCP is to reduce or even eliminate the need for endpoint testing. Before the HACCP concept was developed, many processors depended on endpoint testing to determine if their product was satisfactory. This testing can be very tedious and time consuming. Also, testing can lead to a loss of a portion of the product since some types of testing are destructive (Bauman, 1990). HACCP attempts to reduce endpoint testing by conducting a series of checks throughout the process. At each step in the process, all possible hazards are considered regarding how to prevent them and what actions will be taken if a significant hazard occurs (Mortimore and Wallace, 2000). By the time the product reaches the end of the process, HACCP attempts to reduce hazards to an acceptable level. A third purpose of HACCP is to provide documentation to prove that the process is being conducted as written. Without documentation and records, there is not verification that anything has taken place. According to the FDA (1999), the advantages of HACCP over other safety systems are preventive program and includes, focuses on identifying and preventing hazards from contaminating food, is based on sound science, permits more effective government oversight because record keeping allows

investigators to determine how well a firm is complying with food safety laws over a period rather than how well it is doing on any given day places responsibility for ensuring food safety appropriately on the food manufacturer or distributor .

According to Mayes (1994), “Implementation of HACCP is not a quick ‘back o the envelope’ job done on a quiet afternoon, but it is instead a detailed technical evaluation of a product and process requiring time, commitment, scientific and technical expertise to carry out hazard analyses and establish control and monitoring procedures, and the requisite knowledge, skills and attitude for successful implementation”.

1.7 Microbiology of poultry

The consumption of poultry meat increased worldwide within the last decades (Food and Agriculture Organization (FAO), 1993; McNamara, 1997; Mead 1997). Competition for an increased share of the poultry meat market centers on lowering the price, thus making poultry more attractive for the consumer. Therefore, modern poultry processing requires a high rate of throughput to meet consumer demand. With complete mechanization and automation, the number of slaughtered birds in many processing plants can reach 12,000 birds per h (James *et al*., 2000). However, broilers entering slaughter processing are highly contaminated by microorganisms, including foodborne pathogens such as *Salmonella* and *Campylobacter* spp., and these pathogens tend to be disseminated in the processing plant during processing (Mead *et al*., 1994; Kotula and Pandya, 1995).

1.8 Bacterial Genera involved in Poultry Meat

According to Bensteed (1965) Thornton (1968) Riemann (1969) Buchanan and Gibbons (1974) Cowan (1974) Hobbs and Christian (1974) Hubbert *et al*., (1975) Dugid *et al*., (1978) and Jay (1986) many gram positive and gram negative bacterial genera which were encountered in poultry meat:

1.8.1 The genera of Gram Positive Bacilli

1.8.1.1 *Campylobacter jejuni*

Campylobacter is widely spread in nature and is isolated from wild and domestic animals as well as from the environment. Poultry is a major reservoir of *Campylobacter jejuni*. Many commercial poultry flocks appear to be symptomless carriers of *C. jejuni*, with up to 107.g-1 of gut content being demonstrated in the ileum and caeca of infected poultry and similar levels in the feces (Genigeorgis *et al*., 1986; Mead, 1989; Zottola and Smith, (1990). Some poultry flocks that are negative before slaughter will therefore become contaminated during processing. *Campylobacter* is micro aerophilic with a relative high minimum growth temperature (30°C) and there seems little likelihood of them multiplying in the processing plant or on the raw, processed product (Mead, 1982).

The main problem in processing is that of cross-contamination (Zottola and Smith (1990); Smeltzer, 1981). *Campylobacter* species are more sensitive than many other organisms to the adverse effects of environmental conditions (drying, freezing and cold storage). For this reason, attention has been given to factors influencing the survival of *campylobacter* in processing (Mead, 1982). Although freezing is harmful to Campylobacter, it does not eliminate this organism from poultry. Nevertheless, the contamination rate tends to be higher in fresh than in frozen carcasses. *Campylobacter spp*. are also more sensitive to chlorine than *E. coli*, but are not eliminated from poultry carcasses by immersion chilling in chlorinated water. On the contrary, cooling-water seems to be an important reservoir of this organism: 100-3000 CFU.ml-1 were demonstrated and survival over long periods at low temperatures is possible. Campylobacter was also isolated from air samples as well as equipment (Cunningham, 1987; Mead, 1989; Zottola and Smith., 1990).

1.8.1.2 Bacillus

Bacillus is a genus of the family *Bacillaceae*. It is aerobic, spore-formers in air, dust, soil, water, and on utensils and various foods. Many are as important in the spoilage of many foods held above refrigerator temperature. *B. cereus and B. mesentericus* were species reported to be involved in food-poisoning. The characters of members of the genus are aerobic, Gram-positive endospore producer and on culture media long chains were produced (Mead, 1982).

1.8.1.3 Corynebacterium

Corynebacterium, a genus which is the *Coryneform* group. Members of the genus are found in the intestinal tract of man and animal and had been isolated from spoiling foods of various types. The characteristics of the genus were Gram-positive rod showing granules and cloud-shaped swelling, non-spore formers, mesophilic and psychrophilic, non-motile and non-capsulated bacilli. The species *C. dephtheriae* is milk-borne (Mead, 1982).

1.8.1.4 *Listeria monocytogenes*

Listeria monocytogenes is widely distributed in nature and the environment. These organisms are isolated from soil, vegetation and faeces of humans and animals, with poultry often being contaminated. Studies also indicated that 57% (20 of 35 samples) and 33% (17 of 51 samples) of market poultry, contained *L. monocytogenes,* the organism can multiply at refrigeration temperatures. Data also suggests that *L. monocytogenes* is more heat resistant in meat than *Salmonella*. The necessity of proper hygiene procedures in handling, processing and packaging of poultry is therefore emphasized (Zottola and Smith., 1990).

1.8.2 The Genera of the Gram Positive Cocci

1.8.2.1 Staphyloccus

Staphylococcus is a genus of the family *Micrococcaceae*. It is commonly found in the nasal cavity and skin of man and certain other

animals. The coagulase-positive members of the genus e.g. *Staphylococcus aureus*. Food poisoning from poultry meat caused by *Staphylococcus aureus* is much less common than that due to *salmonellae* or *Clostridium perfringens* (Todd, 1980; Mead, 1982). *Staphylococcus* is important in relation to poultry meat, because it can produce enterotoxins which may cause food poisoning in humans (Notermans *et al.,* 1982). Live poultry carry *Staphylococcus aureus* on skin surfaces and in nasal cavities, but low numbers are also present in the intestinal tract (Todd, 1980; Evans, 1986; Grau, 1986; Mead, 1989). Isolates of Staphylococcus aureus from poultry can be subdivided into human, non-human and intermediate types (Gibbs *et al*., 1978; Mead 1989). It appears that *Staphylococcus aureus* may also be obtained from human sources after hatching and during processing of the carcasses (Gibbs *et al*., 1978; Mead, 1982). Notermans *et al*. (1982) indicated that after processing, contamination of carcasses with this organism increased to >103 g-1 of skin. Defeathering machinery may support the buildup of *Staphylococcus aureus*. Evisceration and chilling are also processing stages which have been incriminated in contaminating carcasses with *Staphylococcus aureus* (Gibbs *et al.,* 1978; Todd, 1980; Mead, 1982; Notermans *et al.,* 1982; Mead, 1989).

1.8.2.2 Streptococcus

Streptococcus is a genus in the family *Streptococaceae*. Some species were reported to be associated with the upper respiratory tract of man and other animals causing scarlet fever and septic sore thorax (Mead, 1982). Others were in the intestinal tract of man and animals. They were described as Gram-positive, catalase-negative cocci producing small colonies within the mesophilic and psychrophilic (Mead, 1982).

1.8.3 The Genera of Gram Negative Bacilli

1.8.3.1 Escherichia

Escherichia is a genus of family *Enterobacteriaceae*. It's described as a coliform of the intestinal tract of man and other animals from which it might be found in soil, water and many other places in nature. *E. coli* and *Aeroactor Aerogens* are known as Gram-negative, short rods, lactose fermenter. *E. coli* was reported as the most important entero-pathogenic coliform and differentiated from *A. aerogenes* by IMVIC reaction. This common lactose fermenting faecal genus shown to have serotypes pathogenic for humans. They are frequently reported in the literature and are known as entero-pathogenic *E. coli* (EPEC). Certain serotypes of *E. coli* produce food-borne disease (Mead *et al.,* 1993; FAO, 1997).

1.8.3.2 Klebsiella

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

1.8.3.3 *Proteus*

Proteus is a genus of the family *Enterobacteriaceae*, and this species is found in the intestinal tract of man and animals. They are Gram-negative, motile, urease -positive. *P. vulgaris* and *P. morganii* produce hydrogen sulphide in abundant quantities, to liquefy gelatin and to swarm on moist agar. Outbreaks of food poisoning were ascribed to *Proteus* (Riemann, 1969; Mead *et al.,* 1993).

1.8.3.4 *Pseudomonas*

Pseudomonas is a genus of the family *Pseudomonadaceae*. The species is widely distributed in nature in soil and water, plants in the intestinal tract of man and other animal. These were found to be the most important bacteria in the low temperature spoilage of food such as meat and poultry. They were motile and non-motile Gram-negative rods producing water-soluble pigment causing metallic sheet. An epidemic involving 409 cases of acute enteritis were reviewed and *P. aeruginosa* was isolated from many patients (Riemann, 1969).

1.8.3.5 *Salmonella*

Salmonella are the main cause of food poisoning from poultry meat (Dougherty, 1976; Todd, 1980). Little is known about the incidence of *Salmonella* in South Africa although figures have been reported by (Bok *et al.* (1986) and (Georgnaras *et al.* 1996). There are many sources from which poultry may obtain *Salmonella*, the main sources being from cross-contamination during breeding, hatching and intensive rearing operations. *Salmonellae* are not part of the normal intestinal microflora of poultry, but are acquired from the farm environment via insects, rodents and birds. Feed is also an important source of *salmonellae* through contamination of various components of the feed mix (Georgnaras *et al.,* 1996). The organisms occur more often in the caecum than in any other region of the gut from where they may be excreted for varying periods, without the host showing any sign of disease (Morris and Wells., 1970; Mead, 1982; Grau, 1986; Silliker and Galois., 1986; Mead, 1989; Zottola and Smith., 1990; Jones *et al*., 1991). *Salmonellas* from one flock can contaminate another, usually during conditions of intensive rearing and when there is inadequate cleaning and disinfecting of the multi-cage transportation Lorries used to convey the birds to the abattoir (Georgnaras *et al.,* 1996). Studies have also shown that live poultry transported from the farm often introduce

Salmonella into the processing plant. Such contamination may result in considerable scattering of *salmonellae* during processing especially in the plucking machines and the scalding tank and may lead to contamination of the final product (McBride *et al.,* 1980; Mead, 1982; Mead, 1989; James *et al.,* 1992).

1.8.3.6 *Shigella*

Shigella is a genus of the family *Enterobacteriaceae*. *Shigella* organisms are Gram-negative rod, non-motile, aerobaic and facultatively anaerobic, catalase postive, oxidase negative, sugar fermenting without gas production and citrate negative (James *et al.,* 1992). *Sh. Flexneri* and *Sh. Sonnei* are the common cause of dysentery in Britain and *Musca domestica* was reported to be the common mechanical vector transferring the organisms from faeces to food. The species of this genus occur in nature, polluted water and the intestinal canal of man where they cause bacillary dysentery. Shigellosis or bacillary dysentery was reported as human disease caused by members of the genus *Shigella* which included four serological distinct species *Sh. dysenteriae, Sh. Flexneri, Sh. Boydii and Sh. Sonnei*. The spread of the infection was shown to be by the faecal-oral route from person to person via the hands or contaminated objects (Mead, 1982; Mead, 1989; James *et al.,* 1992).

1.9 Source of bacterial contamination

Microbial contamination of carcasses is a natural result of procedures necessary to produce retail products from live animals. Contamination of poultry meat products can occur throughout initial processing, packaging, and storage until the product is sufficiently cooked and consumed. Heavy loads of bacteria enter the processing plant with the live bird, and these bacteria can be disseminated throughout the plant during processing. Most of the bacterial contaminants are non-pathogenic and are associated with

meat spoilage(Conner, et al., 2000). However, poultry serve as reservoirs for a number of pathogens including, *Salmonella* serotypes, *Campylobacter jejuni, Listeria monocytogenes, Clostridium perfringens,* and *Staphylococcus aureus*

The Identification and control of aerobic bacteria were reported by Lillard (1990) to increase safety and quality of broiler carcasses. He also claimed that bacterial contamination was reduced significantly by commercial procedures implementing hygienic measures. Control of *enteropathogenic* bacteria was indicated by Zivkovic, *et al.* (1989).

The most important genera of bacteria known to occur in foods were given by Jay 1986. They were 29 in numbers and included *Acetobacter, Acinetobacter, Aeromnas, Alcaligenes, Altramonus, Bacillus, Brochothrix, Campylobacter, Citrobacter, Clostridium, Corynebacterium, Lactobacillus, Leuconstoc, Micrococcus, Moraxella, Pediococcus, Ptoteus, Pseudomonas, Salmonella, Serratia, Shigella, Staphylococcus, Streptococcus, Vibrio and Yersinia*. Their primary sources presented were, soil, water, plants, and plant products, food utensils, intestinal tract of man and animals, food handlers feeds, animal hides, air and dust (Mead, 1982).

Abdella (1993) investigated the aerobic bacteria of carcasses and main edible viscera of poultry slaughtered in the State of Khartoum. He isolated bacteria of the following 20 genera: *Campylobacter, Listeria, Bacillus, Kurthia, Stahylococcus, Streptococcus, Micrococcus, Aerococcus, Escherichia, Erwinia, Klebsilla, Pseudomonas, Salomnella, Shigella, Citrobacter, Proteus, Enterobacter, Edeardsiella and Morganella.*

Most of bacteria found on poultry surfaces were found to consist of flora that were present prior to slaughtering and they were picked up during defeathering, pinning from workers hands and knives, from eviscerating or cooling due to cross 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 the skin and the lining of the body cavities occurred during washing, plucking and evisceration. The authors also stated in their book that sanitation of the housing of the birds before killing had some influence on the numbers of microorganisms on the skin at dressing (Mead *et al.,* 1993).

1.10 Processing in slaughterhouse

The main processing steps in poultry slaughter house are as follows: birds are removed from crates, hung by the feet on shackles on a conveyor, stunned by a low voltage electric shock in a water bath and killed by exsanguinations following slitting of the neck and severing the carotid arteries. They are then scalded, defeathered and washed. Heads, feet and the viscera are removed (Mead *et al.,* 1993; FAO, 1997).

The carcasses are then washed and chilled in cold water or in humidified air. After chilling, the carcasses are further processed or packaged and stored chilled or frozen (McMeekin and Thomas., 1979; Bryan, 1980; Mead, 1982; Grau, 1986; Bailey *et al*., 1987). During each stage of the process, opportunity exists for the contamination of the carcasses with micro-organisms from the environment of the poultry processing plant or by cross-contamination from other birds (McMeekin and Thomas, 1979a). Numbers of bacteria on carcasses surfaces vary considerably at different stages of processing and increases and decreases in numbers have been demonstrated (Thomas and McMeekin., 1980). Defeathering and evisceration are the two stages where bacterial contamination mostly takes place (Mead, 1982; Grau, 1986).

1.10.1 Pre-slaughter Handling and Transportation

For transportation to the processing plant, birds are usually caged in batches. However, stress caused by transport, crowding and exposure to weather conditions may lead to an increased frequency of defecation and discharge of ceacal contents (Mead, 1982; Grau, 1986; Parry, 1989). In the little space available, birds tend to stand in an accumulation of their own droppings. Cages with solid floors used during transportation enable birds to sit in accumulated droppings.

On the other hand, cages with perforated floors allow birds at higher levels to contaminate birds at lower levels (Mead, 1982; Grau, 1986; Mead, 1989). There is evidence that stress occurring during transportation can increase the proportion of birds which are intestinal carriers of *Salmonella* (Mead, 1982). It is therefore usual to starve birds before slaughter to minimize fecal contamination of carcasses during transportation and processing (Anand *et al*., 1989; Mead, 1989). During unloading, it is inevitable that some birds will struggle and flap their wings as they are hung on the shackles, and the results in a considerable scattering of dust and micro-organisms. The only effective control in preventing the spread of airborne contaminants is the complete separation of this area from the rest of the processing plant (Mead, 1982; Mead, 1989).

1.10.2 Scalding

The process of scalding the carcass is used prior to feather removal. This process subjects the carcass to immersion in hot water, facilitating the opening of the feather pores so that the feathers may be removed more effectively. There are two types of scalding, hard and soft. A hard scald, in which the carcass is immersed in water that is greater than 55°C, removes the cuticle (or epidermis) of the skin. If the carcass is immersed in water that is _55°C the cuticle is not removed, and the carcass is soft scalded. Scalding tends to partially remove dirt, fecal material, and other

contaminants found in the feathers. However, these contaminants may be spread to other carcasses through scalder water. Most plants use a countercurrent scalder, in which water for the continuous overflow is fed from the cleanest end of the scalder (that end nearest the picking machines) toward the dirtiest end. This helps to reduce the amount of cross contamination. Tests on scald water have shown that *C. perfringens* and *S. aureus* can be isolated. However, *Salmonella* spp. and *Campylobacter* spp. are usually not isolated (Casey et al., 2010).

1.10.3 Defeathering

While defeathering of carcasses reduces the overall bacterial load via removal of the feather, the process is of major concern to the poultry industry because the modern mechanical process of feather removal can be a major contributor to cross contamination(Casey et al., 2010). This process usually leads to an increase in the number of non-psychotrophic organisms on the individual carcasses, defeathering has also been attributed to an increase particularly in *S. aureus* because the organism becomes embedded in the cracks of the rubber fingers. It has also been attributed to the cross contamination of carcasses by *Salmonella* spp., *Campylobacter* spp., and *E. coli.* This may be due to embedding of these microorganisms in the feather follicles after the feather is removed and before the follicle can reduce in size(Conner et al., 2000)..

1.10.4 Evisceration

During evisceration the opportunity exists for contamination with *Enterobacteriaceae* from the intestinal contents. Careless manual opening of the body cavities and manual evisceration leads to contamination of carcasses, especially when the intestines are cut, or the vent is inadequately loosened (Mead *et al.,* 1993; FAO, 1997).

Cross-contamination can also occur due to workers' hands, evisceration implements and other slaughter equipment (Mead, 1982; Grau, 1986; Mead, 1989). No difference was found between plants using manual evisceration and those with automatic equipment, although automatic evisceration can cause considerable damage to carcasses due to rupturing of the intestines when carcasses in a batch varies in size (Mead, 1989). Aerobic mesophiles on the carcasses usually do not increase significantly during evisceration, but the numbers of *Enterobacteriaceae* and the frequency of contamination with *Salmonella* often increase (Notermans *et al.*, 1980; Grau, 1986). Significant contamination with *Staphylococcus aureus* can occur even though *Staphylococcus aureus* is not detected in the intestinal tract. This contamination comes from sources other than the bird and the contaminating strains also appear to be endemic to the processing plant (Notermans *et al*., 1982). Washing of carcasses after evisceration and before chilling removes organic matter and some of the micro-organisms acquired during evisceration. The visceral cavities also become contaminated during evisceration, especially when the intestines are cut, and it is less easily reached by washing with conventional washing equipment (Notermans *et al.,* 1980; Mead, 1982; Connor *et al*., 1987; Jones *et al.,* 1991). However, strategically sited spray-washers with high-pressure and the use of water containing at least 40ppm available chlorine are effective in reducing the number of bacteria and 70ppm chlorine almost totally eliminated build-up of bacteria (Notermans *et al.,* 1980; Bailey *et al*., 1987; Mead, 1989).

1.10.5 Packaging

The functions of a food package can be divided into four areas: containment, information, convenience, and protection (Barron, 1995).

Containment includes the holding of a product without necessarily protecting it. Holding multiple pieces of chicken parts such as legs, thighs, wings, or breasts allows for them to be sold in various volumes or combinations. Information is both a governmental regulation and marketing tool. The package carries the nutritional labeling, proper handling practices, product information, and identifiers required by law. The package also contains the product price, claims, and cooking suggestions as well as package recycling messages. Convenience is a function of the package. Single serving sizes of sliced meat and microwaveable packages allow for cooking/reheating and consumption of the product in a part of the package. Protection is the most important package function, protecting the product from microorganisms, rodents, dust, external contaminants, humidity, light, and oxygen. The package should also protect the product from tampering and physical damage during handling. Unpackaged meat would quickly dehydrate; therefore the package must prevent moisture loss. Poultry meat having higher pigment concentrations must be protected from loss of the bright red oxymyoglobin color. Products such as ground leg meat and comminuted meat are packaged in films with high oxygen permeability to maintain the oxymyoglobin state. Comminuted meat is packaged in paperboard boxes that allow for oxygen presence and prevent light from contacting the meat surface (Alan, 2001).

1.10.6 Chilling

In many processing plants, the rate of processing is such that there is little loss of heat from the carcasses before it reaches the chilling stage. The deep muscle temperature of the freshly eviscerated carcasses is "30°C and to prevent and limit the growth of spoilage bacteria and pathogens it is necessary that the carcasses must be chilled rapidly and efficiently after evisceration to a keep temperature of below 10°C (McMeekin and Thomas.,

1979a; Mead, 1989). Two methods of chilling are in common use; one involving dry chilling in cold air and the other is immersion of carcasses in ice-chilled water (Mead, 1982; Mead, 1989). Continuous immersion chilling is the most widely used method and comprises one or more units, each consisting of a large tank capable of holding many hundreds of carcasses, through which water flows continuously. The water can flow with or against the direction taken by the carcasses (Bryan, 1980; Mead, 1982). In through-flow systems carcasses move in the same direction as the water flow.

In counter-flow chillers the birds are moved mechanically in the opposite direction to the flow of in-coming water (Mead, 1982). Hygienic operation of immersion chillers requires measures to prevent a build-up of microbial contaminants in the cooling medium and this depends on the water usage and temperature control (Mead *et al.,* 1993; FAO, 1997).

Adequate use of fresh water aids the cooling process and prevents the chiller temperature from reaching a point when bacterial growth becomes a problem (Mead, 1989). The water temperature at the carcasses entry and exit points must not exceed 16°C and 4°C respectively (Mead, 1982). Counter-flow immersion chilling (in which carcasses at the end of the chilling process come into contact with the cleanest water) effectively decreases counts on carcasses and minimizes cross-contamination (Bryan, 1980). Air-chilling, whether as a batch process in a chill room or by continuous air-blast, requires the use of low scald temperatures of ca. 50°C. This is to avoid skin damage and color change of the carcasses (Bryan, 1980; Mead, 1989). Air-chilled carcasses are always likely to have higher bacterial counts than those chilled in properly controlled immersion systems. Several studies have confirmed this supposition, although the differences are relatively small and usually less than 10-fold (Mead, 1989).

Air-chilling is less likely to cause crosscontamination than water immersion, but micro-organisms may circulate in the currents of cold air and usually there is some degree of contact between individual birds in the chiller (Bryan, 1980; Mead, 1989).

1.10.7 Post-Chilling Handling

Bacterial counts can increase after chilling, because of the transfer of micro-organisms during weighing and packaging. Even at this stage contamination with *salmonellae* can occur and therefore, the final product should be frozen or transferred to a chill store without delay (Bryan, 1980; Mead, 1989).

1.11 Drug Sensitivity

Poultry is a major fast-growing source of meat in the world today, representing a quarter of all the meat produced in 2000 (Adeleke and Omafuvbe, 2011). The modern poultry industry can produce market ready broiler chickens in less than six weeks. This accomplishment is done through genetic selection, improved feeding and keen health management practices involving usage of antibiotics as therapeutic agents to treat bacterial diseases in intensive farming systems (Apata, 2009).

Acquired resistance against frequently used antibiotics have been observed since the introduction of these antimicrobial agents in human and veterinary medicine (Smith, 1999). The use of antibiotics is a major factor in emergence, selection and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine (Tollefson and Flynn 2002). The rise in antibiotics resistance have been reported in the past two decade (Kapil, 2004) and antibiotic resistance still remains a global problem today. In intensively reared food animals, antibiotics are administered for therapeutic purpose and as Antimicrobial growth promoters (AMGPs) to the whole flock rather than individuals (Van der

Bogaard and Stobberingh 1999). Resistance to antibiotics can either be naturally occurring for a particular organism/drug combination or acquired resistance, where mis-use of anti-microbials results in a population being exposed to an environment in which organisms that have genes conferring resistance (either spontaneously mutated or through DNA transfer from other resistant cells) have been able to flourish and spread. Hence, the antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their fecal flora contains a relatively high proportion of resistant bacteria (Van der Bogaard and Stobberingh 1999). Resistant strains from the poultry gut readily soil poultry carcasses and when consumed, they alter or affect human endogenous flora (Van der Bogaard and Stobberingh 2001). Gene transfer occurs majorly *in vivo* between gastrointestinal tract bacteria and pathogenic bacteria as identical resistant genes are present in diverse bacterial species from different hosts (Scott, 2002). The test of bacterial isolates were inoculated into nutrient agar and followed by application of the discs impregnated with different antibiotics. Agar disc diffusion method (Baur *et al*., 1996; SFM, 2003) was employed. Antibiotic disc contained the following seven antibiotics Nitrofuratoin (F) - 300µg, Augmentin(AMC)- 30µg, Ciprofloxacin (CIP)- 5µg, Nalidixic acid (NA)- 30µg, Erythromycine (E)- 10µg, Chloramphenicol (C)- 30µg, Gentamicine (CN)- 10µg (Adedeji and Onwenefah, 2013).

Chapter Two
Materials and Methods

2.1 Study area

The swab samples were collected from broiler chickens in one modern slaughter house in Eastern Nile locality, Khartoum state- Sudan.

2.2 Samples collection

A total of 154 swab samples were taken in the period between June to October 2012 from the breast, back, leg and neck after hanging in the slaughter area, after bleeding, after scalding, after washing, after chilling, from slaughtering knives, scalding water, packing machine and hands of the workers(Bryan,1980).The organism were removed from each swab by shaking for a few minutes in 10 ml sterile 0.5% peptone water.

2.3 Bacteriological test

The isolation and identification of bacteria were carried out according to the methods of Barroow and Feltham (2003). The viable count (TVCs) of the isolated microorganism was also used (Harrigan and MacCance, 1976).

2.3.1 Isolation and Identification Procedures

Swab sample were cultured in nutrient agar, nutrient broth, MacConkey agar (MCA) and Blood agar. The plates were incubated at 37^{o}C for 24hrs. Well isolated colonies obtained from agar medium and different broth cultures of Gram-negative and Gram-positive bacteria were constantly sub cultured into agar slants from time to time, incubated at 37^{0}C for 24 hrs. and stored at 40C (Alamin, 2014).

Identification of organisms was based mainly on biochemical tests included: Indole production, Presence of catalase, Acid and gas production, Microscopic and macroscopic examination of morphology and Gram stain. The methods TVC and Identification of the different strains that

used were as described by Harrigan and MacCance, (1966) Barrow and Feltham (1993).

2.3.1.1 Liquid Cultural Media

2.3.1.1.1 Peptone water

Prepared according to Cruikshnk *et al.* (1975). Tengram peptone and five grams NaCl were dissolved by heating in 1000 ml distilled water. The pH was adjusted to 7.2 and the medium was distributed in five amounts in the test tubes and sterilized by autoclaving at 1150 C for 15 minutes under pressure 15lb per square inch. The stock was preserved in the refrigerator.

2.3.1.1.2 Nutrient broth

Contain lab-lemco powder one-gram yeast extract(Oxoid,2006) two grams peptone five grams and sodium chloride five gram. The pH was adjusted at 7.4 approximately. An amount of 13 grams of the dehydrated medium was added to one liter of distilled water. The reconstituted medium was mixed well and distributed in five ml amounts and sterilized by autoclaving at 121^0C for 15 minutes under pressure 15 lb per inch.

2.4 Solid Cultural Media

2.4.1 Nutrient Agar

Nutrient agar was obtained in a dehydrated form (Oxoid,2006) the medium contained, Lab-lemco powder one gram, yeast extract two grams, peptone five grams, NaCl five grams, and agar 15 grams per 1000 ml. PH was adjusted at 7.4 approximately. The medium was prepared by adding 28 grams of dehydrated medium to 1000 ml distilled water and dissolved by boiling and distributed in final containers and sterilized by autoclaving at 115 ^{0}C for 15 minutes under pressure 15 lb per square inch.

2.4.2 Blood Agar

Blood agar was prepared according to Barrow and feltham, (1993). Ten ml sterile defibrinated sheep blood was added to 90 ml nutrient agar which was melted and cooled to 50 ^{0}C. The blood agar after mixed well was distributed (15-20 ml) under flame into sterile petridishes and allowed to solidify at room temperature. The prepared plates were kept in the refrigerator at (4^0 C).

2.4.3 MacConkey's Agar

MacConkey's agar was (Oxoid,2006) contained peptone 20 grams, lactose ten grams, bile salts five grams, neutral red 0.075 grams and agar 12 grams. PH was adjusted at 7.4 approximately. Forty grams of the dehydrated medium were suspended in one liter of distilled water dissolved by boiling, then sterilized by autoclaving at 121 ^{0}C for 15 minutes under pressure 15 lb. per square inch. The medium was dispensed in sterile petri dishes.

2.4.4 Mannitol Salt Agar

Mannitol salt agar (Oxoid,2006) contained: lab-lemco powder one gram, peptone ten grams, mannitol ten grams, sodium chloride 75 grams, phenol red 0.025 grams, and agar 15 grams. An amount of 111 grams of the dehydrated medium was suspended in one liter of distilled water. The mixture was boiled to dissolve completely. Then sterilized by autoclaving at 121 ^{0}C for 15 minutes under pressure 15 lb. per square inch and distributed in sterile Petri dished 15 ml each.

2.4.5 Biochemical Tests

The Enteropluri-test was done as described by the producer company (LIOFILCHEM Bacteriology Products Via Scozia Zona Ind. Le - 64026 Roseto D.A –Italy). An isolated colony from agar medium was picked up by needle of the Enteropluri-test system without penetrating into the agar, afterwards that the needle was inoculated throughout the sectors of the

system. After that the system was incubated at 37C for 24 hours. Finally the change in color in the different sectors was observed for positive reactions and the bacterium was identified by following instructions on codebook. Indole test by using Kovac's reagent (3-4 drops) was added, then positive pink – red color has developed within 15 minutes for E. coli. The catalase test was done by mixing a dense culture with 2 drops of H2O2 and looking for bubbles. The presence of bubbles indicates a positive test and the organisms considered to be Staphylococcus (Barrow and Feltham, 2003).

2.5 Methodology of Viable Bacterial Cell Count

Serial dilutions were used, plating and counting of live bacteria to determine the number of bacteria in a given population was used. Serial dilutions of a solution containing an unknown number of bacteria were made.

The total viable count (TVCs) of the isolated microorganism was carried out according to the method of Harrigan and MacCance (1976). A serial dilution of each sample was made to form (10-1 up to 10-5). Generally the sample is diluted in tenfold increments to make the resulting math simple, this was done by preparing five sterile, labelled beakers from (1) to (5). From this solution 10 ml was pipette into beaker (2) which contains 90 ml of distilled water to yield a total volume of 100 ml to form 10. The process continued until serial dilution of original bacterial suspension in beaker number (5) was made. Each dilution was spread out on a disposable Petri-dish contained a solidified agar medium, MacConkey, s, Nutrient agar, blood agar. Then 0.1-0.2 ml of the dilution was taken out, this was done by sterile bent spreader. Then all plates incubated upside down at 37°C. After 24

hours the number of all colonies on the plate (between 30-300) was counted for each dilution and the mean count was determined. Each colony forming unit represented a bacterium that was present in the diluted sample, therefore the concentration of viable bacteria per millilitres in initial sample can be calculated and expressed in CFU /ml. Bacterial identification The morphology of colonies on agar media were examined microscopically, smears were made from clean slides fixed with heat and subjected to Gram stain and examined under oil immersion. In addition to that, the identification has also been based mainly on biochemical tests (Barrow and Feltham, 2003). Biochemical tests The Enteropluri-test was done as described by the producer company (LIOFILCHEM Bacteriology Products Via Scozia Zona Ind. Le - 64026 Roseto D.A –Italy). An isolated colony from agar medium was picked up by needle of the Enteropluri-test system without penetrating into the agar, afterwards that the needle was inoculated throughout the sectors of the system. After that the system was incubated at 37C for 24 hours. Finally the change in colour in the different sectors was observed for positive reactions and the bacterium was identified by following instructions on codebook. Indole test by using Kovac's reagent (3-4 drops) was added, then positive pink – red colour has developed within 15 minutes for E. coli. The catalase test was done by mixing a dense culture with 2 drops of H2O2 and looking for bubbles. The presence of bubbles indicates a positive test and the organisms considered to be Staphylococcus (Barrow and Feltham, 2003).

2.6 Antibiotics Sensitivity Disk

The bacterial isolates were tested for resistance to 8 antibiotics produced by MAXIDISC (Maxi care medical Laboratories Ltd., Nigeria). These were: gentamyin (Gen), amoxicillin (Amc) , ciprofloxacin(Cip) , vancomycin (Van), erythromycin(Ery), penicillin(P), chloronphenicol (C), floramphenicol(Fc).

This testing was performed using the standard disc diffusion method (Musa *et al.*, 2019).

The antibiotics susceptibility pattern of the isolates was interpreted using Progressive Diagnostics Manufacturers (PDM) Interpretative Chart (Hemen *et al.*, 2012).

2.7 Statistical Analyses

The collected data were analyzed with SPSS software (Statistical Package for the Social Science, version Il.5SSPS Inc and Chicago, IL, USA). All bacterial counts were converted to $\log_{10}$ CFU / Cm^{-2} for analysis and ANOVA were performed. Statistical significance was set at P value of <0.05.

Chapter Three
Results

Statistical significant differences were not observed at *p-value* of ≤0.05 when the total viable count (TVC) was compared between the processing steps. As shown in Table 3.1, the highest TVC level reported from breast (8.15) was at after evisceration, from back (5.56) was at after evisceration, from leg (6.60) was at after bleeding, and from neck (9.50) was at after evisceration.

Table 3.1: Comparison of the TVCs (log_{10}cfu/g or cm^2) between the samples taken from the processing steps in Khartoum.

Sites	Processing steps (log_{10}cfu/g or cm^2)							*P.Value*
	A	B	C	D	E	F	g	
Breast	5.35	5.56	3.62	6.48	8.15	5.54	3.45	NS
Back	3.34	4.60	5.38	5.29	5.56	2.13	5.46	NS
Leg	5.50	6.60	4.27	4.33	4.56	4.66	3.62	NS
Neck	6.38	5.65	4.61	5.57	9.50	6.54	5.54	NS

A = Hanging and slaughtering area,B = after bleeding,C = after scalding,D= afterdefeathering,E = after evisceration, F = after washing,g = after chilling, NS = not significant at *p-value* ≤ 0.05, and * = significant at *p-value* ≤ 0.05

Five species of bacteria, namely: *E. coli*, *Salmonella, Proteous, Pseudomonas* and *Staphylococcus* were isolated and identified from the 7 processing steps under investigation as shown in Table 2. The most detected bacteria were *E. coli* (n = 46, 27.4%) and least detected was Proteous (n = 9, 5.4%). *E. coli* and Salmonella were most detected in Hanging and slaughtering area (n = 12, 50.0% and n = 10, 41.7%), Proteous and Pseudomonas in after evisceration (n = 3, 12.5% and n = 5, 20.8%), and Staphylococcus in after scalding (n = 10, 41.7%).

Table 3.2: The number of bacteria isolated from operational points of the carcasses in broiler carcasses in abattoir, Khartoum state.

Processing steps	**Isolated bacteria**				
	E. coli	***Salmonella***	***Proteous***	***Pseudomonas***	***Staphylococcus***
At hanging (n=16)	0 (0.0%)	6 (37.5%)	2 (12.5%)	0 (0.0%)	8 (50%)
After bleeding (n=16)	4 (25%)	4 (25%)	0 (0.0%)	0 (0.0%)	8 (50%)
After scalding (n=16)	2 (12.5%)	2 (12.5%)	2 (12.5%)	0 (0.0%)	10 (62.5%)
After defeatherin g (n=16)	10 (62.5%)	2 (12.5%)	0 (0.0%)	0 (0.0%)	4 (25%)
After evisceration (n=16)	8 (50%)	4 (25%)	0 (0.0%)	0 (0.0%)	4 (25%)
After washing (n=16)	8 (50%)	2 (12.5%)	2 (12.5%)	2 (12.5%)	2 (12.5%)
After chilling (n=16)	6 (37.5%)	8 (50%)	0 (0.0%)	0 (0.0%)	2 (12.5%)
Total (n=112)	38	28	6	2	38

A = Hanging and slaughtering area,B = after bleeding,C = after scalding,D= afterdefeathering,E = after evisceration, F = after washing,and g = after chilling.

Table 3.3 showed the TVC levels of slaughter knife, scalding water, and packing machine as well as the isolated bacteria. The TVC levels were 4.43, 2.54, and 2.59, respectively, from the three points while *E. coli* (n = 3, 16.7%) was the most detected bacteria at slaughter knife, *Pseudomonas* (n = 4, 22.4%) at scalding water, and *Staphylococcus* (n = 2, 11.1%) at packing machine.

Table 3.3: TVCs (log_{10}cfu/ml or cm^2) and type of isolated bacteria in slaughter knife, scalding water, and packing machine in Khartoum

Processing steps	Count	Sites				
		E. coli	Salmonella	Proteo us	Pseudomonas	Staphylococcus
SlaughterKnife	4.43	3 (16.7)	2(11.1)	0(0.0)	0(0.0)	2(11.1)
Scaldingwater	2.54	0 (0.0)	0 (0.0)	2(11.1)	4 (22.4)	0(0.0)
PackingMachine	2.59	1(5.6)	0 (0.0)	0(0.0)	0(0.0)	2 (11.1)

Hands of workers were investigated at some processing steps including H & S Area, during evisceration, cutting, and packing (Table3.2). Statistical significant differences at *pvalue* of ≤0.05 were not observed between the

TVC levels recorded from Hands of workers at the different the processing steps but the highest level was 9.49 recorded at during evisceration. Staphylococcus (n = 7, 38.9%) was the most detected bacteria at H & S Area, Pseudomonas and Staphylococcus (n = 2, 11.1%) at During Evisceration, E. coli (n = 4, 22.4%) at Cutting, and Staphylococcus (n = 4, 22.4%) at Packing.

Table3. 4: TVCs (log_{10}cfu/cm^2) and detected bacteria from hands of workers in Khartoum

Point	Hands of Workers					
	Count	E. coli	Salmonella	Proteous	Pseudomonas	Staphylococcus
H & S Area	5.54	0(0.0)	1(5.6)	0(0.0)	0(0.0)	7 (38.9)
During Evisceration	9.49	0(0.0)	1(5.6)	1(5.6)	2 (11.1)	2 (11.1)
Cutting	5.48	4 (22.4)	0(0.0)	1(5.6)	0(0.0)	1(5.6)
Packing	3.58	1(5.6)	1(5.6)	0(0.0)	0(0.0)	4 (22.4)

As summarized in Table3.5, E coli was mostly affected by Gen/10 and Cip/5 (2.5 mm) and least by P/10 (0.8 mm), Salmonella was mostly affected by Gen/10 (2.4 mm) and least by C/30(0.5 mm), Proteous was mostly affected by Gen/10 and Amc/30 (2.4 mm) and least by C/30 (0.7 mm),Pseudomonas was mostly affected by Gen/10 (2.3 mm) and least by C/30 (0.5 mm), and Staphylococcus was mostly affected by Gen/10 (3.0 mm) and least by C/30 and P/10 (0.9 mm).

Table 3.5: Susceptibility of the detected bacteria at the different processing steps, slaughter knife, scalding water, packing machine, and hands of workers to several anti-biotic(GEN/10= gentamycin,AMC/30=amoxicillin,Cip/5=ciprofloxacin,Va/30= vancomycin,Fc/10=floramphenicol, E=erythromycin,P=penicillin,C=chloronphenicol) in Khartoum.

Antibiotic	Staph	E coli	Protius	Salmonella	Pseudomonas
Gen/10	3.0	2.5	2.4	2.4	2.3
Amc/30	2.8	2.4	2.4	2.2	2.0
Cip/5	2.5	2.5	2.2	2.3	2.1
Va/30	2.3	2.4	2.3	2.2	2.1
Fc/10	1.9	1.8	1.5	1.5	1.5
E/10	1.5	1.6	1.5	1.4	1.2
P/10	0.9	0.8	0.8	0.7	0.8
C/30	0.9	0.9	0.7	0.5	0.5

Chapter Four
Discussion

Statistical significant differences were not observed at *p-value* of ≤0.05 when the TVC was compared between the processing steps, from table no (3.1) the highest level of contamination observed after evisceration and the value was 9.50 and 8.15 (neck and breast) and 6.60 for leg after bleeding , but Kabour (2011) reported ,TVCs 7.69±2.6 in legs, 7.49±1.6 in backs and 8.38±2.1 in breasts after defeathering. Dougherty (1974) and Cox *et al*. (1995). Abu-Rwaida *et al*., (1994) and Geornaras *et al*., (1997) reported that 100% of birds were contaminated with *Salmonella spp.* immediately after bleeding and Lillard (1989) showed that the incidence of *Salmonella spp*. was fell to 11.9% after defeathering, then increased to 14.3% after evisceration.

In table no (3.2), the most detected bacteria was *E.coli* (n = 46, 27.4%) In hanging and slaughtering area(h&s area), and the lowest was *proteous* (n = 9, 5.4%) and the critical point was after evisceration, but Mohamed-Noor (2012), founded *Escherichia coli* (11.11%) after defeathering and after evisceration, the role of *Proteus sp.* in foodborne infections has been a debate over the past years, despite its potential as a pathogen. *Proteus sp*. is associated with food deterioration and hence its presence cannot be directly related to infections, but as a contaminating agent (Biranjia and Latouche, 2016). *Proteus sp*. is involved in the decomposition of carcasses and can be found in faeces, putrefied meat, sewer water, suppurating wounds, and others (Cardoso *et al*., 2006).

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* (Georgnaras *et al*., 1997; Göksoy *et al*., 2004).

The presence of *E. coli* in chickens is an indication of poor hygienic practices in abattoirs or trading areas. A wide variety of both plant and animal foods are potential sources of *E. coli* contamination, especially chickens. *E. coli* has also been found worldwide in poultry meat products (Adzitey et al., 2011). The prevalence of this bacterium in both poultry and poultry products differs across different parts of the world. The prevalence rate of *E. coli* has been recorded to be as high as 98% in India (Sharma and Chattopadhyay ,2015). In Sudan, the prevalence of E. coli was as high as 57.8% (Sharma and Chattopadhyay ,2015), whilst in Morocco it has been reported to be 48.4% (Cohen *et al*.,2007) and 16% in Nigeria (Adzitey *et al.,* 2011). In table (3.3) the E. coli (n = 3, 16.7%) was the most detected bacteria at slaughter knife, the *Pseudomonas* was (n = 4, 22.4%) at scalding water and (n= 2, 11.1) was the percent of *Staphylococcus* in Packing Machine, Gabeer. *et al* (2012) reported that, the *E. coli* and *Salmonella spp*. *E,coli* was isolated in all operational points After Defeathering, Evisceration, spray first washing, spray second washing and After chilling, whereas *Salmonella spp*. was isolated in two operational process After Evisceration and After spray first washing.

That is fit with Abu-Rwaida *et al*. (1994) found increased numbers of bacteria following scalding and defeathering. When birds are immersed in the scalding tank, dirt, fecal material, and other surface contaminants are removed.

Hands of workers were investigated at some processing point including hanging and slaughtering Area(H&S), during evisceration, cutting, and packing.

Table no (3.4) Statistical significant differences at p-value of ≤0.05 were not observed between the TVC levels recorded from Hands of workers at the different the CCPs but the highest level was 9.49 recorded at during evisceration. *Staphylococcus* (n = 7, 38.9%) was the most detected bacteria at H and S Area, and 4(22.4) in packing area, and the higher level in cutting area was *E. coli4*. (22.4).

Bailey *et al*. (1987) reported that although low levels of *Staphylococcus aureus* were routinely isolated, *Salmonella spp*.

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* (Geornaras *et al*., 1997; Göksoy *et al*., 2004). In this study the mean TVCs obtained from chicken carcasses after slaughter with feathers, after scalding, after defeathering, after evisceration, and scalding water were higher than those reported by Göksoy *et al.* (2004).

Pesewu *et al.* (2018), founded that, the isolated Bacteria from the raw broiler meat samples investigated were *Proteus mirabilis* (26.9%), *Proteus vulgaris* (25.1%), *Klebsiella sp.* (23.4%), *Salmonella sp*. (10.8%), coagulase negative *Staph aureus* (9.2%), and *Escherichia coli* (4.8%). Various enteric bacteria including species of *Proteus* (86.7%) and *Klebsiella* (6.7%) have been found in chicken faeces (Pesewu *et al.*, 2018)

Pre-washed chickens, post evisceration, had lower levels of contamination at 35% compared to post-washing with higher contamination levels at 65%. These data strongly suggest that the observed increase in contamination levels after washing is likely to be due to the increased use of reused water

during processing of the chicken carcasses. Consequently, good water quality and a close monitoring of water are needed throughout the processing line (Mpundu *et al*., 2019).

Multidrug resistant bacterial of animal origin may spread into the human population by direct contacts and through food from animal source (Kolář *et al.* (2002).

Conclusion

1. The highest rates of contamination occurred on the carcass after evisceration.
2. The organisms isolated from poultry in each point were E. coli , Salmonlla , Proteous , Pseudomonas , Staphylococcus .
3. All isolated bacteria in this study were sensitive to gntamycin 10 mg
4.The elimination of contamination sources by practicing good hygiene and sanitary measures will reduce the occurrence of microorganisms.

Recommendations

1. Poultry carcasses can be contaminated at any proseccing points, but applying of standered methods of hygiene leading to safe broiler meat for consumption.
2. Appropriate method of HACCP should be applied during slaughtering operation, all worker's must be informed to know how to keep they hands clean and how to manipulate the carcasses.
3. This aminoglycoside (gentamycin) is considered to be the drug of choice for treatment of bacterial infections.

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