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Research

Bacterial Contamination Of Chicken Carcasses At Abattoir in Khartoum State-Sudan

التلوث البكتيري لذبائح الدواجن بمسلخ في ولاية الخرطوم

By

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Dedication

To my parents and sincerely husband and my sons ahmed, Abubaker, Omar & my sisters and brothers

Acknowledgments

First of all, my thanks and praise are due to almightily Allah, and then my sincere thank goes to: my supervisor Prof Mohammed A. Abdulla, of department of preventive Medicine and public Health College of Veterinary Medicine, Sudan University of Science and Technology, for his guidance help and kindness with me, to all staff member of Alnasoor slaughter house for co-operation and co-ordinate research project in HACCP system application. Science and faithfully thanks are due to my small family for their tremendous support, encouragement and practice.

Abstract

The present study aimed to give an available, easy, safe and effective solution to reduce bacterial load in post-slaughter house contamination on the carcasses of broilers by applying the Hazard Analysis Critical Control Point (HACCP) System at the AL-nasoor Slaughter house in Khartoum.

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.

From four Critical Control Points (CCPs) on the broilers processing line. eighty samples were collected and examined to determine bacterial contamination.

The obtained results showed that the concerned bacteria were determined in different operation (Four CCPs) and sides (legs, backs, breast) respectively, There were no significant differences between these operations ($P < 0.05$). Whereas in washing the TVCs were 5.80 ± 0.40.

Bacteriological findings at each Critical Control Point (CCPs) gave evidence that post-slaughter contaminations constituted hazard, i.e. I detect in this study acceptable in this study acceptable contamination, which contained two types of bacterial genera, staphylo coccus, E. coli, that affect the quality and safety of poultry meat produced commercially at Khartoum State, in order to take safe and effective solution to reduce bacterial load.

ملخـص الأطـروحـة

هذه الدراسة تهدف إلى إإعطاء الحلول الأمنة والسهلة والمتاحة والفعالة لتقليل التلوث الجرثومي بعد الذبح، على ذبائخ الفراريج، من خلال تطبيق نظام تحليل المخاطر، عند نقاط التحكم الحرجة بمسلخ النسور فى ولاية الخرطوم.

من اربع نقاط التحكم الحرجة (CCPs) على خط تصنيع الفراريج، ثم جمع 80 عينة وتم فحصها لتحديد التلوث الجرثومي.

مخاطر نقاط التحكم الحرجة (HACCP) هو برنامج لتحديد والسيطرة على الأخطار الميكروبيولوجية المرتبطة بتجهيز الدواجن، وقد تم تطبيقها على صناعة الدواجن لتحسين الجودة الميكروبيولوجية للذبائح والحد من الأخطار الميكروبيولوجية من المزرعة للإستهلاك.

وأظهرت النتائج التي تم الحصول عليها فى النقاط الربعة (CCPs)، وكانت لا توجد إختلافات كبيرة بين هذه النقاط ($P<0.05$). وعن الغسل كانت (0.40 – 5.80).

البكتريا التى وجدت فى كل نقطة حرجة أعطة دليلاً على أن مرحلة ما بعد الذبح تشكل خطراً على جودة وسلامة لحوم الدواجن، وفى هذه الدراسة وجدنا تلوث مقبول يحتوى على نوعين من البكتريا الإستاف والأيكولاي، بحيث يمكن أن تؤثر فى جودة وسلامة لحوم الدواجن المنتجة تجارباً بولاية الخرطوم اذا لم يتبع الحلول الأمنة والفعالة للتقليل منها.

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Introduction

The consumption of poultry meat increased worldwide within the last decade's food and agriculture organization (FAD, 1993, Me, Namara, 1997 Meal 1997 competition for an increased share of the poultry meat market centers on lowering the price, Thus making poultry more attractive for the consumer, there for, modern poultry processing requires a high rate of through put to meet consumer demand with complete mechanization and automation the number of slaughtered birds in many processing plant can reach 12,000 birds per h (james etal., 2000).

Food safety was identified as a high priority area in the 2001 - 2005 world organization for animal health (o/E) strategic plan member countries of o/E considered that the organization should be more active in issues of public health and consumer protection and that this should include more involvement in the area of disease or pathogens transmissible through food (Droppers, 2006). Meat and meat productions are of particular importance regarding food borne illnesses food borne pathogens can be introduced to food during processing storage and preparation from some other raw agricultural products head berg Mac Donald & Osterholm.

Poultry meat can be easily contaminated with microorganisms, due to many factors, as nutrients, high water activity and neutral PH. These characteristics are favorable to the development of microorganism, which are intrinsic of poultry or microorganisms that come from external sources during the processing.

Health chickens ready for processing harbor a tremendous amount and variety of bacteria. These bacteria are present on the surface of feet, feathers, skin and also in the intestines, during processing, a high proportion of these organisms will be removed, but further contamination can occur at any stage of the

processing operation.

The procedure for converting alive, health bird into a safe and wholesome poultry

product provides many opportunities for micro- organisms to colonize on the surface of the carcasses. During the various processing operations, opportunities exist for the contamination of the carcass from the environment.

The process in the plant itself, contamination via knives, equipment, the hands of workers and also by cross-contamination from carcass to carcass. Some processing operations encourage an increase of contamination or even multiplication of contaminating organisms.

As a result, the microbial population changes mainly from positive rools and micro cocci on the outside of the live chicken to from micro- organisms on the finished product (Baryan 1980: Thomas and me meekin, 1980 Roberts, 1982; ... 1986; Bailey et al; 1987; Conor et al; 1987; Ban wart, 1989; Meal, 1989).

Poultry processing has a number of unique features which make control of microbial contamination more difficult than the processing of any other conventional meat animal, among them is rapid rate of processing in some plants, a condition which favors the spread of micro-organisms.

The carcass must be kept whole throughout the process and the small opening in the abdomen without breakage, to minimize contamination of the carcass with intestinal organisms after de-feathering.

The skin provides a complex surface with many holes which are capable of trapping bacteria (Mead, 1982; Grave, 1986; Meal 1989).

The micro-organisms are widely distributed over the carcasses under normal circumstances and are spread over the skin during scalding and de-feathering and on the inner and outer surfaces during evisceration and further processing (Baileg et al; 1987).

Effort should be made to prevent the build-up of contamination during de-feathering and evisceration is therefore of great importance (Mc Meekin and Thomas; 1982; Anand et al; 1998). Spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surface under the skin, the nature and rate of attachment of the micro-organisms depend upon several factors included the bacteria
involved and their concentration and also the conditions under which attachment occurs, namely, PH, temperature and contact-time. It was also found that pseudomonas strains attach to meat surfaces more rapid than any other bacteria (Furstenberg - Eden, 1981).

The structure of the skin also has crucial influence on attachment of bacteria. The organism adhere by way of flagella and vibrate and cannot easily be using, especially after a delay.

The bacteria also show that mesophilic bacteria are more heat - resistant when attached (Barnes ex al; 1973; Green, 1974; firstberg- Eden; 1981; Thomas and Mc Meekin, 1981; Faber and Idziak, 1984; Lillard, 1985).

The skin serves as a barrier to micro-organisms that might be otherwise contaminated the underlying muscle and therefore the deep muscles are normally free of bacteria (Bryan, 1980; Meal, 1982).

The few bacteria found in the deep muscle are of types that can only multiply slowly or not at all at low temperatures. The important microbiological changes take place on the surfaces of the carcasses. It appears that some parts of the carcass are more favorable than others for bacterial growth, depending of the type of the muscle and PH.

Studies conducted over the last three years show that the sites most heavily contaminated are the neck skin and less frequently on the back and the area around the vent. Fewer organisms are found around the breast. Legs, and under

the wings. Acientobacter and Alteromonas grow better in leg muscle where PH is 6.4 to 6.7 than in breast muscle where PH is 5.7 to 5.9 pseudomonas spp. Can grow well at both PH ranges (Patterson, 1972; Barnes etal; 1973; Green, 1974; Mc Meekin and Thomas; 1979 a; Bryan 1980; Thomas and Mc meekin; 1981; meal, 1982; Gill 1983; Grau, 1986 Anaud et al: 1989). The presumable reason for the neck skin being the most heavily contaminated is that the washings from the rest of the carcass run down the neck while the carcass hangs on the conveyor (Patterson, 1972; Connor et al; 1987).

So, the implementation of H ACCP (Ha2ard Analysis and Crittica Control Points) in poultry industry is extremely important, because it involves the constant monitoring of all steps of the process, aiming the food safety of final product industries must implement this food safety program to serve both internal and external market (jmene2 et al; 2002; Meal, 2004; Galhardo et al; 2006).

Objectives

General Objective:

To assess measures of poultry meat safety in slaughter house by applying the principle of selected hazard analysis Critical Control Point.

Special Objectives:

1. To determine bacterial number (Total Viable counts) on poultry meat.
2. To isolate and identify bacteria at different check points.
3. To assess the feasibility of using the (HACCP) system in slaughter houses in Sudan to achieve good practice of poultry meat hygiene.

Chapter one

Literature Review

1.1 History of the poultry industries in Sudan

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 A. A. Makelmenjeri. Late in 1963 the American Aid Programme established Kuku Poultry Farm. Breeds such as White Leghorn, Fayoumi, Rhode Island Red, New Hampshire and Light Sussex were introduced into the Sudan.

During the period from 2001 - 2005 a significant increase in the number of farms, as a result 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 chick, 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 bird yielding 1.3 million kilograms of meat lssawi, 1977). He also stated that the Government was well aware of the importance of developing poultry in the Sudan and hence laid down in 1976 the promotion of Agriculture Investment Act, As a result 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 bird 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 at 8 week 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 and Nagla (1998).

1.2 Conception of quality and safety of poultry meat and HACCP:

Microbiological criteria establish the acceptability of a product based on presence or absence of microorganisms, quantity of toxins per mass unit, volume and, area or batch. The microorganisms included in a criterion must be relevant, as pathogens, indicators, or spoilers (Food and Agriculture organization (F AO 1997). The microbiological quality of chicken meat can be determined by its sanitary conditions, contamination during processing, storage conditions, transportation and commercialization. However, contamination occurs easily in the first stage of slaughtering (Galhardo et aI., 2006).

Before evisceration process, carcasses must be washed in shower with pressured water, since the whole carcass must be washed including feet.

After the washing, chicken are submitted to evisceration, which can be automated or not. In this step, viscera tearing and the direct contact with contaminated surfaces must be avoided. The automated stage involves cloacae extraction, abdomen cutting, and evisceration. After this stage, sanitary inspection occurs and viscera are manually removed, and the edible giblets (heart, liver and gizzard) are separated to be chilled. Windpipe and crop are removed mechanically, and carcasses are finally washed both external and internally, before passing through the pre-chilling system (Brasil, 1998). Pre-chilling process decreases carcass temperature after the process of evisceration and washing.

This system has stainless steel tanks with cold water added with ice cubes (both

are chlorinated at 1 ppm maximum), where chickens are immersed.
They are called pre-chiller and chiller. These tanks have a continuous carrier thread, while water and ice are being renewed every time, and its flow is against chicken carcass flow. At pre-chiller system, the temperature of water cannot be over 16°C; at chiller tank, the temperature must be at 4°C maximum, and chickens must be until. 7°C in the end of this process.
These parameters contribute to the reduction of any microorganisms that could be found III the poultries (Brasil, 1997; and 1998). The evisceration process can cause tearing of intestine, gall bladder or crop, contaminating chicken surface with their contents. Affected chicken carcasses are reprocessed, or in extreme cases, condemned, but the reprocessing or the condemnation delays the operation of the processing plant and increases the cost of producing a quality product (Northcutt et al., 1997).
When visible contamination occurs, skin with fecal, gastric or bile waste is trimmed. After it, knives must be washed in potable water and immersed in hot water in order to disinfect the blade. This procedure aims the use of carcasses for cutting and processing. However, if the knife is not appropriately washed, removing skin can contaminate the meat, and some microorganisms could be transferred from contaminated skin to meat.
Also skinless carcasses pass through the showers and washing machine, aiming to be cleaned internally and externally before is chilled (Northcutt et al, 1997; Brasil 1998). According to Food Safety and Inspection Service (FSIS), since 1978 the food industries can proceed the non-contamination by many methods, and washing is allowed for chickens as an alternative to knife trimming. Studies have presented that the first method is equally effective in removing fecal contamination (Food Safety and Inspection Service 1997).
The overall bacterial levels of freshly processed poultry carcasses are influenced by the time of feed withdrawal before slaughter (Bilgili, 1988), transport and crating time, outside air temperature and plant temperature ,

processing steps (Mead et al., 1993), plant temperature control, and hygienic practices in the plant (Barnes, 1976; Bailey et al., 1987; Mead, 1989). Although it is impossible to ensure the complete absence of pathogens from broilers, the risk of food borne disease can be reduced substantially by minimizing their numbers.

Mead (1989) summarized the reasons why controlling microorganisms in poultry processing is difficult as: 1) the rapid rate of production keeps the birds in close proximity throughout processing, 2) limitations in the design of processing equipment, including that used in scalding, defeathering, and evisceration, 3) the difficulty of washing the abdominal cavity effectively after evisceration when the carcass remains whole, 4) retention of water by skin, which tends to entrap bacteria in the crevices and feather follicles, (Notermans and Kampelmacher, 1974; McMeekin and Thomas, 1978; Lillard, 1989). During processing, most of the Gram-positive bacteria originating from live birds are removed and replace by a heterogeneous population mainly composed of Gram negative bacteria, including Pseudomonas, flavobacteria, Acinetobacterl Morexella and Enterobacteriaceae (Thomas and McMeekin, 1980; Mead, 1989).

The HACCP system, as it applies to food safety management, uses the approach of controlling critical points in food handling to prevent food safety problem. The system, which is science based and systemic, identifies specific hazard and measures for their control to ensure the safety of food.

HACCP is based on prevention and reduces the reliance on end-product inspection and testing. The RACCP system can be applied through the food chain from the primary producer to the consumer. Besides enhancing food safety, other benefits of applying RACCP include more effective use of resources, savings to the food industry and more timely response to food safety problems.

1.3 History of HACCP

RACCP has become synonymous with food safety. It is a worldwide-recognized systematic and preventive approach that addresses biological, chemical and physical hazard through anticipation and prevention, rather than through endproduct inspection and testing.

The RACCP system for managing food safety concerns grew from two major developments. The first breakthrough was associated with W.E.

Deming, whose theories of quality management are widely regarded as a major factor in turning around the quality of Japanese products (TQM) systems which emphasized a total system approach to manufacturing that could improve quality while lowering costs.

The second major breakthrough was the development of the RACCP concept itself.

The RACCP concept was pioneered in the 1960s by the Pillsbury Company, the United State Army and the United State National Aeronautics and Space Administration (NASA) as a collaborative development for the production of safe foods for the United State space programmed. NASA wanted a zero defects programmed to guarantee the safety of foods that astronauts would consume in space. Pillsbury therefore introduced and adopted RACCP as the system that could provide the greatest safety while reducing dependence on end-product inspection and testing. RACCP emphasized control of the process as far upstream in the processing system as possible by utilizing operator control and/or continuous monitoring and techniques at critical control points. Pillsbury presented the RACCP concept publicly at conference for food protection in 1971. The use of HACCP principle in the promulgation of regulations for low-acd canned food was completed in 1974 by the United State Food and Drug Administration (FDA). In the early 1980s, the HACCP approach was adopted by other major food companies.

1.3.1 Principles of HACCP

For the purposes of this study, the following seven principles that are the basis of the HACCP system have been sourced from the Codex Alimentarius Commission. Report of the 29th Session of the Codex Committee on Food Hygiene (1996) (Alinorm 97113A) "Hazard Analysis and Critical Control Point (HACCP) System and Guidelines for its Application" .

1.3.1.1Principle 1

Conduct a hazard analysis.

1.3.1.2 Principle 2

Determine the Critical Control Points (CCPs).

1.3.1.3 Principle 3

Establish critical limits.

1.3.1.4 Principle 4

Establish a system to monitor control of the CCP.

1.3.1.5 Principle 5

Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

1.3.1.6 Principle 6

Establish procedures for verification to confirm that the HACCP system is working effectively.

1.3.1. 7 Principle 7

Establish documentation concerning all procedures and records appropriate to these principles and their application. MAP Regulatory Authority (Meat and Seafood) HACCP Steering Group Amendment 9: (August 2004).

1.3.2 Advantages of HACCP

The HACCP system, as it applies to food safety management, uses the approach of controlling critical points in food handling to prevent food safety

problem. The system, which is science based and systemic, identifies specific hazard and measures for their control to ensure the safety of food. HACCP is based on prevention and reduces the reliance on end-product inspection and testing.

The HACCP system can be applied through the food chain from the primary producer to the consumer. Besides enhancing food safety, other benefits of applying HACCP include more effective use of resources, savings to the food industry and more timely response to food safety problems.

HACCP enhances the responsibility and degree of control at the level of the food industry. A properly implemented HACCP system loads to greater involvement of food handlers in understanding and ensuring food safety, thus providing them with renewed motivation in their work.

Implementing HACCP does not mean undoing quality assurance procedures or good manufacturing practices already established by accompany, it does, however, require a revision of these producers as part of systemic approach and for their appropriate integration into the HACCP plan.

The application of the HACCP system can aid inspection by food control regulatory authorities and promote international trade increasing buyer's confidence.

Any HACCP system should be capable of accommodating change, such as advances in equipment design, changes in processing procedures or technological development.

1.3.3 Application of HACCP

While the application of HACCP to all segments and sectors of the food chain is possible, it is assumed that all sectors should be operating according to good manufacturing practices (GMPs) and the Codex General Principle of Food Hygiene. The ability of an industry segment or sector to support or implement the HACCP system depends on the degree of its adherence to these practices. The successful application of HACCP requires the full commitment and

involvement of management and the workforce. It requires a multidisciplinary approach which should include, as appropriate, expertise in agronomy, veterinary health,microbiology, etc. according to the particular situation. The application of the HACCP system is compatible with the implementation of TQM system such as the ISO 9000 series.

However, HACCP is the system of choice in the management of food safety within such systems.

1.4 Bacteriology of Poultry Meat:

Drewnaik et a!., (1954) found that there was a buildup of bacteria on the skin of chicken during dressing and evisceration. They also found that the procedures after dressing which include washing with pressurized sprays od water decreases the bacteria present on the a skin of poultry.

According to FAa (1984 - 1988), it was stated that for human diet poultry meat had been considered a valuable source of protein. Nevertheless, the composition as substrate was claimed to provide the need for growth and multiplication of many genera of spoilage and disease producing bacteria (Jay, 1986). These varieties of bacteria might perpetuate if poultry meat was not properly prepared and hygienic practice were not followed (Jensen, 1954).

The rearing of large numbers of table poultry for slaughtering at high through-put was also found to contribute in failure of reducing the bacterial contamination of carcasses (Price and Schweigert, 1971.).

The significance of bacterial contamination in poultry meat was organoleptic changes or spoilage and human illness due to infection or intoxication. In this respect, they described meat as an ideal culture medium for many organisms. The reasons were its high moisture, richness nitrogenous protein, plentiful supplement with minerals and accessory growth factor, presence of fermentable carbohydrates and its favorable pH

for most microorganisms. 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 the meat which included moisture content and pH the availability of oxygen and temperature (Frazier and West off, 1978)

1.5 Microbiology of poultry:

Contaminants may be micro-organisms that cause spoilage of the product or organisms of public health significance. Pathogens associated with poultry are Salmonella, Staphylococcus aurous, Clostridium perfringens and Escherichia coli. Listeria monocytogenes and Campylobacter jejuni have also been isolated from poultry. Spoilage bacteria most frequently associated with poultry are Pseudomonas spp., Acinetobacter, Moraxella, Alteromonas putrefaciens, Aeromonas spp., Corynebacterium, Flavobacterium, Micrococcaceae and Enterobacteriaceae. Poultry is a common vehicle of foodborne illness (Bryan, 1980; Todd, 1980; Smeltzer, 1981; Brown and Baird-Parker., 1982; Mead, 1982; Roberts, 1982; Ralph and Tompkin., 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).

1.6 Bacterial Genera involved in poultry meat:

According to Bensteed, (1965) Thornton, (1968) Riemann, (1969) Buchanan and Gibbons, (1974) Cowan, (1974) Hobbs and Christian, (1974) Carter, (1975) Lahellic et al., (1975) 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:

1.6.1 The genera of Gram-negative bacilli included:

1.6. 1.1 Escherichia,

Escherichia, a genus of family Enterobacteriaceae. Its described as a coliform of the intestinal tract of man and other animals from which it might be found in

soil, water and many other places in nature. E. coli 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.

1.6.1.2 Klebsiella

Klebsiella, a genus of 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 faecal samples from outbreak of poisoning involves 30 students (Riemann, 1969).

1.6.1.3 Proteus

Proteus, a genus of the family Enterobacteriaceae. This species is found in the intestinal tract of man and animals. They are Gram-negative, motile, urease - positve. 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.

1.6.1.4 Pseudomonas

Pseudomonas, 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 nonmotile Gram-negative rods producing water- soluble

pigment causing metallic sheet. An epidemic involving 409 cases of acute enteritis was reviewed P. aeruginosa was isolated from many patients (Riemann, 1969).

1.6.1.5 Salmonella

Salmonella are the mam cause of food poisonmg 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 (Geomaras 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.

Salmonellas are not part of the normal intestinal micro flora of poultry, but are acquired from the farm environment via insects, rodents and birds.

Feed is also an important source of salmonellas through contamination of various components of the feed mix.

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 also when there is inadequate cleaning and disinfecting of the multi-cage transportation lorries used to convey the birds to the abattoir.

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).

1.6.1.6 Shigella

Shigella, 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. 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.

1.6.2. The genera of the Gram-postive cocci included:

1.6.2.1 Staphyloccus,

Staphyloccus, a genus of the family Micrococcaceae. It is commonly found in the nasal cavity and skin of man and certain other animals. The coagulasepositive members of the genus (e.g. Staphylococcus aureus).

Food poisoning from poultry meat caused by Staphylococcus aureus is much less common than that due to salmonellas or Clostridium perfringens (Todd, 1980; Mead, 1982). Staphylococcus is important in relation to poultry meat, because it can produce enterotoxins which may cause food poisoning in humans (Notermans et al., 1982). 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 aI., 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 > 1 03 g-1 of skin. Defeathering machinery in particular 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).

1.6.2.2 Streptococcus:

A genus of 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. 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.

1.6.3 The genera of Gram-positive bacilli:

1.6.3.1 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 aI., (1986) ; Mead, (1989); Zottola and Smith., (1990). Some poultry flocks that are negative before slaughter will therefore become contaminated during processmg. Campylobacter is micro aerophilic with a relative high mmnnum growth temperature (30°C) and there seems little likelihood of them multiplying in the processing plant or on the raw, processed

product.

The main problem in processing is that of cross-contamination (Zottola and Smith (1990) ; Smeltzer, (1981). Campylobacter spp. is more sensitive than many other organisms to the adverse effects of environmental conditions (drying, freezing and cold storage). For this reason, attention has been given to factors influencing the survival of campylobacter's in processing.

Although freezing is harmful to Campylobacter, it does not eliminate this organism from poultry. Nevertheless, the contamination rate tends to be higher in fresh than in frozen carcasses. Campylobacter spp. are also more sensitive to chlorine than E. coli, but are not eliminated from poultry carcasses by immersion chilling in chlorinated water. 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).

1.6.3.2 Bacillus

Bacillus, 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.

1.6.3.3 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 described 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.

1.6.3.4 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, respectively, contained L. monocytogenes. L. monocytogenes can multiply at refrigeration temperatures. Data also suggests that L. monocytogenes is more heat resistant in meat than Salmonella. The necessity of proper hygiene procedures in handling, processing and packaging of poultry is therefore emphasized (Zottola and Smith., 1990).

1. 7 Sources of bacterial contamination of poultry meat:

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.

Abdella, (1993) investigated the aerobic bacteria of carcasses and mam 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 processmg 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.

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. These genera included:

Acinetobacter, Bacillus, Citrobacter, Micrococcus, Proteus, Pseudomonas, Serratia and Treptomyces.

The hazard of air borne contamination of foods was indicated by Gregry (1961). He claimed that pathogenic organisms might spread in air by food handlers during sneezing or coughing and deposit on meat surfaces.

Bryan (1978) and Jay (1986) considered food handlers to be important source of contamination. The micro flora on their hands and outer garments generally reflect the environment and the habits of the individuals. This flora consisted of

genera found on any object handled by the individuals in the addition to those from water, dust and soil. There were several genera of bacteria specially associated with the hand and nasal cavities and mouth, the most important of which were Micrococcus and Staphylococcus.

A related factor to contamination was the way of holding of feet and heads of the fowl on the slaughter line (Jay, 1986).

Heavy contamination could be reduced (Robert, 1987) if equipment were adequately cleaned and sanitized at short intervals. Hudson and Mead (1989) reported in their study that poultry carcasses acquired listeria mainly via contaminated surfaces and equipments.

In their microbiological survey, (Schuler and Badenhop (1972) found that the packing materials might also represent an important source of contamination.

Aian slaughter processing plants bacterial cell per em square.

Jay (1986) mentioned that the environment of poultry slaughter-house contained several genera of bacteria more than in the soil, water or other places. Among these were Bacteroides, Escherichia, Proteus, Salomnella, Shigella, Staphylococcus and streptococcus.

Hoop and Ehrsam (1987) concluded from their epidemiological investigations that Compylobacter infected broiler flocks resulted from inadequate protection and that the slaughtering process was responsible for contamination of poultry.

Abdel Salam (1986) investigated the prevalence and properties of Clostridium perfringenes in broilers in the Sudan.

Kraft, et. al (1966) reported that the microbial flora of fresh poultry consisted largely of Pseudomonas and other closely related Gram-negative bacteria as well as Corynebacterium, yeasts and other organisms.

Psychrotrophic bacteria were studied by Lahellic, et. al. (1975). They did their studies on 5920 strains isolated from chicken carcasses. They found that bacteria of the genus Pseudomonas constituted 30.5 percent, Acinetobacter 22.7 per cent, Corynebacterium 12.7 per cent with yeast, Enterobacteriaceae and

other in lower numbers.

1.8 Poultry Meat Hygiene:

Banwart (1981) indicated **III** his book that food microbiologist was concerned with the biochemical reaction of micro-organisms in foods.

These reactions would result in spoilage, public health hazards and fermentation production. He also added that determining the type and factors affecting their multiplication were important to develop a system that could be used for their control.

According to Nickerson and Sinkey (1974) holding eviscerated poultry allowed bacteria to grow on both the skin and flesh portions producing slime and causing off odour. The common bacteria on the skin of poultry after processing were found to belong to Pseudomonas. Also during holding at the refrigerator the bacterial flora were chiefly Pseudomonas.

The storage life of eviscerated poultry depended on the extension of contamination after eviscerating and cooling and the temperature at which the finished product was stored. They also stated that eviscerated poultry held at 100°C or below was spoiled mostly by bacteria of the genus Pseudomonas and at a lesser degree yeasts, also Alcaligenes and Flavobacterium.

The microorganisms on or ill poultry meat were of much significance because they were found responsible for deterioration and health hazards (Forrest, et al, 1975). With regard to spoilage, Frazier, and Westhoff (1978) stated that bacteria were the chief cause of spoilage and that bacteria from the intestine were the primary sources of such organisms": Barnes et al., (1978) stated that the length of time of an air chilled chicken or turkey carcass could be kept without spoilage depending on number of factors, the most important being the storage temperature. They remarked that at 50C carcasses develop slight off odour in an average of seven days, Their results indicated the importance of actual refrigeration temperature on the shelf-life of the carcass.

Gracey (1981) clarified that the spoilage ofa drawn poultry was caused by the

bacteria normally found in the intestine, particularly in the caecum and occurred where birds were inadequately stored before slaughtering and inadequately cooled after plucking.

Banwart (1981) explained that smce fresh animal products were perishable, they should be chilled and stored in ice or refrigerator (0-40 DC). Psychrotrophic at this low temperature became dominant and primary cause of spoilage . The organisms most often involved with spoilage of refrigerated fresh meat and poultry were of the genus Pseudomonas.

He also stated that chilled meat spoilage was evidenced by off-odour and slime due to Pseudomonas, Acintobacter and Alcaligenes and the temperature of the meat was the most important factor that determined the predominant micro flora and the resultant spoilage.

At a temperature of 50°C or higher, thermophilic bacteria could grow causing spoilage. Example of which were the genera Bacillus and Clostridium.

At intermediate temperature (15-25 DC) the main spoilage organisms were mesophilic clostridia.

Low temperature spoilage was evidenced on the surface due to Gram- negative bacteria primarily genera Pseudomonas, Aeromonas, Alcaligene, Acintobacter and Moraxella. Anand. et al (1989) pointed out that heavy contaminants of poultry carcasses in the processing line included staphylococci and coli forms.

The Centre for Disease Control (CDC) (1976) reported data complied about biological hazards of foods. They considered them the most important, because in also stated in the recent years attention had been paid to the role of heat-resistant strains of Clostridium perfringenes.

1.9 Influence of processing on poultry:

The main operations in processing poultry 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. 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., 1979a; Bailey et al., 1987; Bryan, 1980, Mead, 1982; Grau, 1986). 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).

1.9.1 Pre-slaughter handling and transportation:

For transportation to the processing plant, birds are usually caged inbatches. However, stress caused by transport, crowding and exposure to weather conditions may lead to an increased frequency of defecation and discharge of ceacal contents (Grau, 1986; Mead, 1982; 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 in order to minimize faecal 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 this 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).

1.9.2 Scalding

Carcasses are scalded to loosen the feathers by immersion in a hot water tank, at either 50 - 52°C (soft scalding) or at 56°C to 60°C (hard scalding) (Bailey et aI., 1987; Mead, 1989). During scalding micro-organisms on the skin and feathers and in the faeces of the birds are washed from the birds and continually released into the water of the scald tank.

Aerobic plate counts of scald water however, are usually less than 5x104 cfu ml-l of scald water (Mulder and Veerkamp., 1974; Bryan, 1980). The survival of Enterobacteriaceae and mesophiles is higher at low scald temperatures of 50°C to 54°C than at higher temperatures (Grau, 1986; Anand et aI.,1989). At a scald temperature of 61°C, reductions of more than 1000-fold can be obtained, whereas at scald temperatures of 53°C to 55,5^{0}C the counts are reduced by10 to 100-fold (McBride et aI., 1980; Notermans et al., 1980; Grau, 1986).

The accumulation and survival of micro-organisms in the scald tank during processing is influenced by the temperature of scalding and the rate at which fresh water is added (Mead, 1982; Bryan, 1986; Bailey et al., 1987). The great reduction in counts during scalding and the absence of Pseudomonas indicate that scald water contamination plays a relative minor role in spoilage of chicken carcasses (Bailey et al., 1987).

Scald temperatures have little effect on the spores of Clostridium perfringens in the water (Mead, 1982; Bailey et al., 1987). Evidence also indicates that the shelf-life of carcasses is reduced by scalding at temperatures above 58°C. This can be attributed to the fact that scalding at about 58°C - 60°C (hard scalding) and above, followed by mechanical plucking results in removal of the outer epidermal layer (cuticle), whereas scalding at 52°C - 53°C (soft scalding) does not. The cuticle free skin of the carcasses serves as a more suitable substrate for spoilage organisms and in particular Pseudomonas (Bryan, 1980; Bailey et al., 1987).

1.9.3 Defeathering:

During defeathering there is a considerable scattering of micro-organisms from carcass to carcass and also from the defeathering equipment itself. The warm, moist conditions under which these operations take place also favour microbial growth. There are two aspects to the contaminating effect of defeathering.

One arises from the extensive aerial scattering of micro-organisms in the vicinity of the machines, and is due to their mechanical action (Mead, 1989). It is therefore necessary to ensure complete separation of the plucking and scalding area from the clean areas of processing (Zottola and Smith., 1990; Mead, 1989).

The other aspect of defeathering hygiene is the nature of the machines themselves, and their sitting next to the scald tank, which helps to maintain a warm moist environment suitable for microbial growth. The rubber "fingers" used to remove the feathers harbour micro-organisms and are not easily cleaned and disinfected (Mead, 1982; Grau, 1986).

Micro-organisms can persist in cracks and other imperfections even after vigorous cleaning (Gibbs et al., 1978; Grau, 1986). Up to 106 Staphylococcus aureus cm-2 can be found on the rubber "fingers" of defeathering machines and treatment with 100ppm chlorine for 30min may reduce the counts by only a tenfold (Gibbs et al., 1978).

The counts of both aerobic mesophiles and psychrotrophs on poultry skin can increase during defeathering and also the numbers of Enterobacteriaceae (Lahellec and Colin., 1979; Thomas and McMeekin., 1980).

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

This cuticle-free skin serves as a very suitable substrate for spoilage organisms and the organisms become trapped in the skin follicles and folds (Thomas and McMeekin., 1980; Grau, 1986; Connor et al., 1987; Mead,

1989).

1.9.4 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.

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 particular 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).

1.9.5 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.

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). Airchilling, whether as a batch process in a chill room or by continuous air-blast, requires the use of low scald temperatures of ca. SO°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 cross-contamination 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).

Chapter two

Materials and Methods

2.1 Sampling Carcasses Surfaces of Broilers:

The method that used as described by Harrigan, (1976) for total viablecount (TVC). Identification and isolation of the bacteria was done according to Barrow and feltham, (1993). With a sterile metal template 10 cm2 area of the thigh region on the broiler carcasses was outlined and was swabbed vigorously with sterile cotton gauze wrapped around the end of a flat swab stick.

The steps of processing of the birds at the Al-rasoor slaughter shown in Figure (1). As observed, the birds were scald and slaughtered.

Carcasses were defeathred together in one group, after scalding at 50 DC. Hock cutting, plucking and evisceration were carried out. The head and neck were removed and the carcasses were cooled -rapidly in air-chilling rooms after being sprayed. At this stage the giblets were inserted in the cavity of each carcass which was packed in a labeled plastic bag. The carcasses at all these stages were moving on the line by the use of motors. Evisceration was done manually. The processed output of poultry meat was distributed on the same day to the retail outlets.

The organisms were removed from each swab by shaking for few minutes in ten ml of sterile 0.5 per cent peptone water. The collected swabs of each carcass were marked, numbered and transported promptly on ice to the laboratory.

Following the HCCP system (Bryan, 1992) the 75 swabs from 25 carcasses were collected at random at the five critical control point (CCPs) determined on the broilers processing line (Table 1). The first CCP was after defeathering. The second was after evisceration. After spray the washing was the third point. after chilling the fourth point.

Samples were collected over a period of one month. Collection was carried out every two weeks. Each time three samples were taken from each CCP on

the processing line of broilers in the slaughter-house of Al- Nasoor slaughter Khartoum state.

On the surface of the carcasses at three sites (leg, back and breast). About 80 Swabs were collected randomly for quantitative and qualitative analysis of bacteria. The organisms were removed from each swab by shaking for few minutes in 10 ml of sterile 0.5 percent peptone water.

Isolation .and Identification procedures: Isolates of test organisms were obtained from four CCPs from slaughterhouse of Al-Nasoor slaughter, using prepared nn'rient agar, nutrient broth, Macf.onkey agar (MCA) and Blood Agar. The plates were incubated at 370C 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 370C for 24hrs and stored at 40C.

Identification was based mainly on the followings:

(i) Indole production **(ii)** Presence of catalase **(iii)** Haemolysis on blood agar **(iv)** Acid and gas production **(v)** Microscopic and macroscopic examination of morphology **(vi)** Gram stain. The methods TVC and Identification of the different strains that used were as described by Harrigan and Macr.ance, (1966). Barrow and feltham, (1993).

Table (1): Distribution of 80 samples collected from broilers carcasses on processing line AI-Nasoor slaughter

Numbers	Critical control point	Numbers of samples
1.	After defeathering	20
2.	After evisceration	20
3.	After spray washing	20
4.	After chilling	20

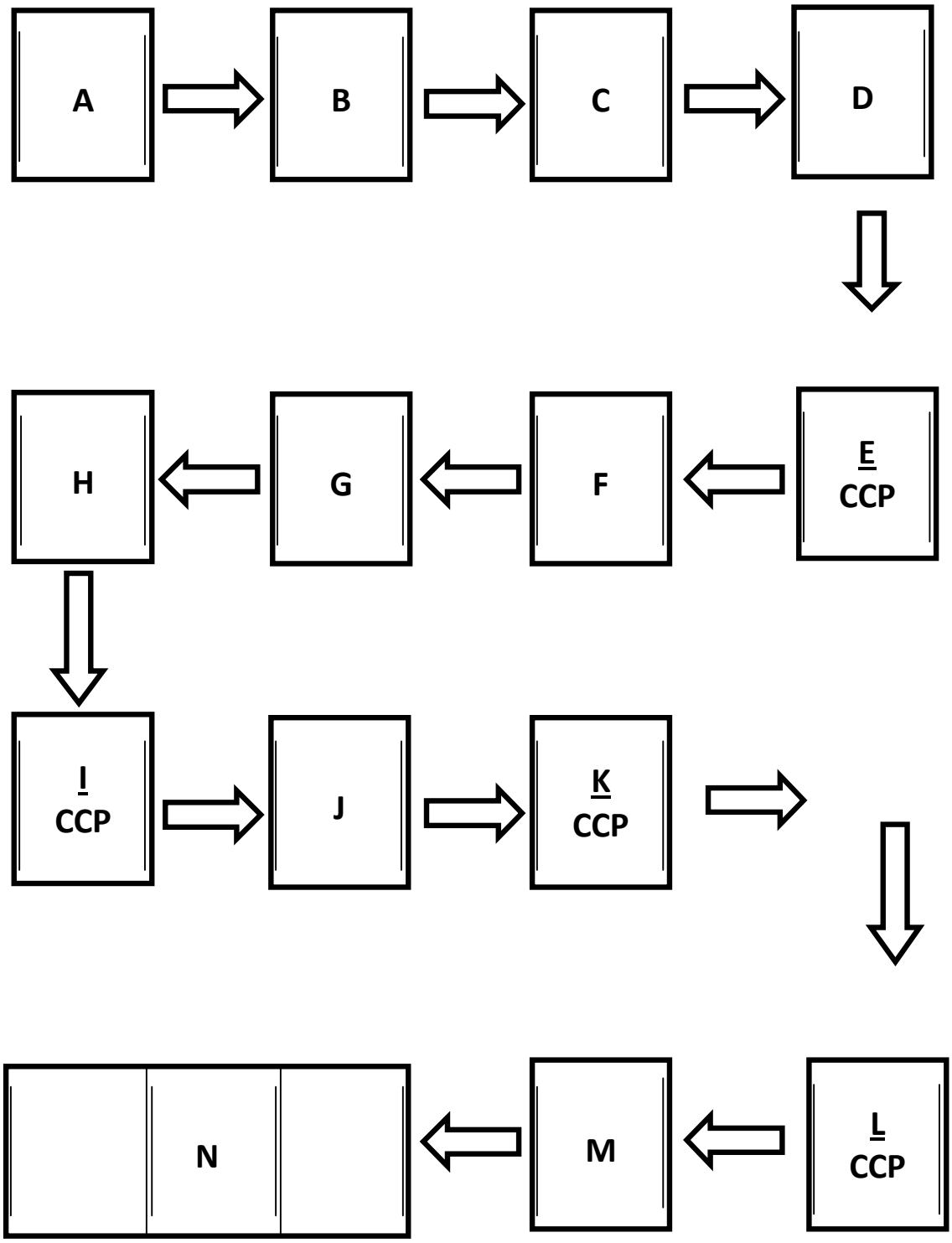

Figure (1) Flow plant operations for processing of broilers at Al-nasoor Slauphter and including CCP

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

Key of the Figure (1)

A- Stunning.

B- Killing and bleeding by cutting the jugular veins.

C- Collection of blood. The conveyor travels through a blood collection tunnel at a preselected travelling speed.

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

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

F- Washing. The defeathered carcasses recerve a spray wash pnor to evisceration.

G- Opening of the carcass by cutting manually. H- Inspection of the viscera.

1- Evisceration, removal of head, feet and viscera.

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

K- First washing to remove blood and to loosen tissues.

L- Chilling of the carcasses in a water bath.

M- Grading, weighing and packing.

N- Chilling anp freezing.

2.2 Liquid Cultural media:

a. Peptone water was prepared according to Cruikshnk., et al (1975). Ten gram peptone and five grams NaCI 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.

b. Nutrient broth. (Oxoid*) contained Iab-lemco powder one gram yeast extract two gram peptone five gramsand sodium chloride five gram. pH was adjusted to 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 1210C for 15 minutes under pressure 15 lb per inch.

2.2.1 Solid Cultural media:

2.2.1.1 Nutrient agar

Nutrient agar was obtained in a dehydrated form (Oxoid*) the medium contained, Lab-lemco powder one gram, yeast extract two grams, peptone five grams, NaCI five grams, and agar 15 grams per 1000 ml. pH was adjusted to 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 1150C for 15 minutes under pressure 15 lb per square inch.

2.2.1.2 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°C. The blood agar after mixed well was distributed (15-20 ml) under flame into sterile Petri dishes and allowed to solidify at room temperature. The prepared plates were kept in the refrigerator.

2.2.1.3 MacConkeys Agar

MacConkeys agar of (Oxide*) contained peptone 20 grams, lactose ten grams, bile salts five grams, neutral red 0.075 grams and agar 12 grams. pH was adjusted to 7.4 approximately. Forty grams of the dehydrated medium were suspended in one liter of distilled water dissolved by boiling, then sterilized by autoclaving at 121°C for 15 minutes under pressure 15 Ib per square inch. The medium was dispensed in sterile Petri dishes in * Oxide Laboratory Products, London.

2.2.1.4 Mannitol Salt Agar

Mannitol salt agar (Oxide*) contained: Iab-lemco powder "'one gram, peptone ten grams, mannitol ten grams, sodium chloride 75 grams, phenol red 0.025

grams, and agar 15 grams. An Rl TIOunt of in 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°C for 15 minutes under pressure 15 lb per square inch, and distributed in sterile Petri dished 15 ml each.

2.3 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 number of bacteria in the original solution was determined by counting the number of colony forming units and comparing them to the dilution factor.

Each colony forming unit represented a bacterium that was present in the diluted sample. The numbers of colony forming units (CFU's) are divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria per mL that were present in the original solution.

2.3.1 Serial dilutions:

Five small, sterile test tubes were prepared labeled 1 through 10 and then 4.5 ml ofM9 salts was added to each test tube. An M9 salt is a physiological buffered minimal medium that contains inorganic salts but no carbon source. Bacteria do not grow in this media but remain in a state of stasis until the diluted cells are plated on media containing a carbon source.

0.5 ml of the original solution was pipette into test tube 1. Bacterial suspension was mixed thoroughly (using the vortexes on each bench) before proceeding to the next step. 0.5 ml of the diluted bacterial suspension was withdraw using from the first test tube a clean pipette and pipettes that into the second test tube. Continual in this fashion until serial dilution of original bacterial suspension into test tube 5 was made. In test tube the bacteria was diluted 10 fold, a 1: 1 0

or 1 x 10'-1 dilution, in test tube 5 was the bacteria diluted from the original tube to obtain a 1 x 10-5 dilution.

Below is the mathematical reasoning for performing the serial dilutions**:**

Tube 1 contains 4.5 ml of sterile media; 0.5 ml of the undiluted bacterial suspension was added to yield a total volume of 5.0 ml.

Tube 2 contains 4.5 ml of sterile media; 0.5 ml of the 1: 10 diluted bacterial suspension was added to yield a total volume of 5.0 ml.

2.3.1.1 Lab 1. Plating the serially diluted cells:

The following dilutions were made: 1 x 10-1, 1 x 10-2, 1 x 10-3, 1 x 10-4, 1 x lOS. Were cultured in TSA plates and incubated at 37°C. After sterilizing the stick, the hockey stick was used to spread the bacterial suspension evenly over the entire surface of the plate. The plate was allowed to dry. This process was done with the remainder of the bacterial dilutions. All the plates were taped together and incubated, upside down, at 37°C for 24 hours.

2.3.1.2 Lab 2. Counting colony forming units and calculating the amount of bacteria in the original solution:

For each dilution, the number of colony forming units on the plates was counted. Typically numbers between 30 and 300 are considered to be in the range where one's data is statistically accurate. Alternatively, if the numbers are evenly distributed on the surface of the plate. If the number of CFU s on the plate is below 10, the number of CFU s has to be recorded but was not used in

the calculations.

T=Trial

Calculation of Average, bacteria/ml Total viable counts (log 10 CFU cm- 2).

Dilution factor	**Numbers of bacterial colonies (CFUs)**					**Avg # CFU**	**Avg # bacteria/ml**
	T1	**T2**	**T3**	**T4**	**T5**		
10-1 1:							
10-2 1:							
10-3 1:							
10-4 1 :							
10-5 1 :							

Chapter Three
Results

As shown in Table 1, there was no statistically significant difference at (p> 0.05). The TVC revealed the highest contamination level after washing *5.80±OAO* loglO cfu /cm"' whereas the lowest contamination level recorded after chilling 5.76±0.381oglO cfu/cm_2.

Table 1 Mean and standard deviation of total viable count of bacteria at different operational points:

Number	Operation point	TVC Log_{10} CFU/Cm^2	Significant difference
1	After defeathering	5.78±	N.S
2	After evisceration	5.77±	N.S
3	After washing	5.80±	N.S
4	After chilling	5.76±	N.S

There were noa significant difference Between these operational points, N.S. Not significant(P > 0.05).

Isolation and identification of bacteria at different operational points under investigation revealed 2 species of bacteria (Table2), which were, after defeathering E colii 4.08%),Staphylo coccus. (26.53%) , after evisceration Ecoli (14.29%), Staphylo coccus (12.24%) after washing, Ecoli(l0.20%), Staphylo coccus (14.29), and After chilling E.coli (8.16%) , Staphylo coccus (10.20%).

Table 2: Isolated bacteria at different operational points:

Number	Operation point	Isolated Bacteria				Total N (%)
		E. Coli		Staphylo Coccus		
		Frequency	%	Frequency	%	
1	**After defeathering**	2	4.08	13	26.53	15 (30.61)
2	**After evisceration**	7	14.29	6	12.24	13 (26.53)
3	**After washing**	5	10.20	7	14.29	12 (24.49)
4	**After chilling**	4	8.16	5	10.20	9 (18.37)
5	**Total**	18	36.73	31	63.27	49 (100)

Chapter Four

Discussion

It is obvious that the variability in microbial counts (especially after washing) indicate the need for use of prerequisite programs. The reduction of the microbial contamination in this study (Table 3) is in agreement with Rahkio and Korkeala (1996) who said that the enforcement of hygienic practice such as regular disinfection of working tools and worker hands is important in reducing the microbiological contamination of carcasses, John *et al.,* (2000) reported the reduction of bacterial contamination during slaughtering after using a degree of sanitation.

Another study by Jeffery (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. Such control measures should include an extensive education programs for proper hygiene and improvement of managements.

Both methods (trimming and washing) for reducing contamination can be applied, aiming to the use of chicken for cutting and processing. So, immersion pre-chilling system is the determinant to the decreasing of number of microorganisms, as long as it is following all legislation requirements. If the chicken carcass is only washed (without the trimming) it can be commercialized intact with quality assurance. Economically, to keep the carcasses with skin is more interesting for the company.

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.

The microbial count on either the carcass depends on various factors. These are the initial count on carcasses, the ambient temperatures, personal hygiene, the efficiency of applied sanitary programmes. Beside changes in the water supply and quality, levels of cleaning of the carcass surface and the general management procedures applied throughout the carcass production Nortije et aI., (1990).

From public health point of view, the most elaborate hygienic precautions in the slaughterhouse can largely be modified by subsequent poor handling of the meat. Little or no control anywhere along the lines i.e. slaughterhouse, Market and restaurant, usually means the onset of a human disease (Ahmed, 2004).

The presence of Escherichia coli as a contaminant of fresh meat which is reported here has also been reported by several workers. Orner (1990) and Ahmed (2004) suggested faecal contamination as a cause of the large numbers of isolates of E. coli they recovered. However, as based on isolates from knives of workers in this study, the presence of E. coli in fresh meat can be attributed to carcass contamination with the gastrointestinal contents during the 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), Hussein (1987). The highest contamination levels recorded in the point of washing in all sites (flank, Hind leg and Shoulder) may be due to unclean management during the washing, this is in agreement with (Ali, 2007) who that reported the highest level of contamination with regard to the critical control point was found in washing point.

Conclusion

1. The lowest rates of contamination occurred in critical control points were found to be after chilling.
2. The highest rates of contamination occurred on the carcass after washing.
3. The organisms isolated from poultry were E. coli and staphy – Coccas.
4. The elimination of contamination sources by practicing good hygiene and sanitary measures will reduce the occurrence of microorganisms.

Recommendations

1. Appropriate methods of HACCP should be applied during slaughtering operations.
2. It's important to use adequate water and disinfectant.
3. Designing slaughtering lines so as to make hygienic working possible is evidently very important.
4. Application of poultry inspection programs so as to reduce the risk of food borne illness.
5. Extensive education programs for proper hygiene and improvement of managements should be adopted.

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